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*EXTRACTION OF COLLAGEN FROM CONNECTIVE TISSUE BY NEUTRAL SALT SOLUTIONS**

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One of the most challenging biological problems concerning the connective tissue relates to the mechanisms by which collagen is synthesized. Presumably, precursors are formed within the connective-tissue cells and transformed into the organized fibrils in extracellular space. Some information concerning this problem has been obtained in electron microscope studies of collagen-forming connective-tissue cells in cultures and in sections.[1] However, in such preparations structures identified as collagen must have axial periodicity characteristic of this protein. Extremely thin collagen particles whose structure may be beyond the resolution obtainable in such preparations would be either missed or unrecognized as collagen. It has thus far been impossible to demonstrate morphologically the step between the intracellular synthesis of the collagenous protein and its appearance extracellularly in the form of characteristically striated collagen fibrils. The missing link is believed to be the biological "monomer" or collagen particle which presumably is synthesized in the cell and transformed into fibrils in the environment of the extracellular space.

Previous in vitro studies of the precipitation of collagen fibrils have been carried out in acid solutions of collagen.[2, 3] However, physical-chemical studies of such solutions, particularly in the presence of citrate buffer,[4] indicate that the collagen exists as long chains of protofibrils or thin bundles thereof, thousands of angstrom units long and having particle weights of the order of 10^6–10^7.[5] Previous studies by the authors[6, 7] suggested the existence of a type of collagen particle having a length of the order of 2,000 A and being very thin, possibly one or a few polypeptide chains in cross-section. This particle was given the name "tropocollagen."[7] Whether or not tropocollagen is present in the tissues in the dispersed state as a biological precursor of the fibrils is yet to be determined.

In 1951 Highberger, Gross, and Schmitt[3] demonstrated that mildly alkaline phosphate buffers could extract from fresh whole connective tissues soluble collagenous material which, after further manipulation, could be transformed to

characteristic collagen fibrils. The possible relationship of this material to precursors of the fibrils was evident.

Studies, described below, were begun on the properties of collagen extracted in its isoelectric range and on mechanisms involved in its precipitation from solution. During the course of this work several papers appeared by Harkness *et al.*,[8, 9] who, using procedures previously described,[3] demonstrated that the collagen thus extracted had a higher glycine turnover rate than that of any of the proteins of the skin and, indeed, higher than that of the blood proteins. They also showed that, although the glycine turnover of acid-extracted collagen ("procollagen") was somewhat higher than that of the residual collagen fibrils, it was not high enough to indicate an early precursor stage. Possibly the acid-dissolved material derived from freshly formed collagen fibrils.

It seems probable that the collagen extractable from connective tissue by neutral or slightly alkaline solutions of phosphate and other salts is present in the ground substance rather than in the organized fibrils, because (1) purified collagen swells little and dissolves hardly at all in the pH range 5–10.5 (Highberger,[10] Bowes and Kenten[11]); and (2) electron microscope examination of fibrils after salt extraction shows that most of the fibrils are relatively normal in appearance, whereas after extraction by acid or acid buffers (such as in the extraction of the so-called "procollagen" of Orekhovitch) the structural organization of the fibrils is badly disrupted.

MATERIALS AND METHODS

The corium of fresh[12] 3–7-day-old calf skin was cut into strips and coarsely ground in a meat grinder with chips of dry ice to avoid excessive heating. Portions were taken for dry weight and collagen estimation. Except where noted, all steps were performed at 0°–5° C. Aliquots of wet tissue, each weighing 50 gm., were extracted for 18 hours on a shaking machine with 50 or 100 ml. of salt solutions (see Table 1). The extraction media included serum ultrafiltrate. In most cases only one or two extractions of the same tissue sample were carried out. In some experiments the tissue was extracted four times, and each extract was treated separately. In one instance the extraction was carried out 24 times with 0.15 *M* phosphate, pH 8.0 ($\Gamma/2 = 0.45$). The experiment eventually had to be discontinued because of excessive bacterial growth.

The extracts were separated from the tissue by centrifugation in the Model L Spinco preparative centrifuge at 80,000 g for 1 hour and then passed through fine sintered glass filters. The filtrates were usually amber- or red-colored, water-clear, and somewhat viscous.

Aliquots of each extract were dialyzed against repeated changes of cold distilled water. The fluffy precipitates which formed were separated by centrifugation, washed in water in the centrifuge, and lyophilized. The supernates were also lyophilized. All transfers and lyophilizations were performed as carefully as possible to avoid loss of material. Precipitates were examined in the electron microscope. Glycine was determined by the method of Christensen, Riggs, and Ray[13] and hydroxyproline according to Martin and Axelrod's modification[14] of the method of Neuman and Logan[15] on lyophilized samples dried *in vacuo* at 108° C.

TABLE 1

COLLAGEN CONTENT OF SALT EXTRACTS OF CALF CORIUM*

EXTRACTANT	Γ/2	pH	PRECIPITATE		SUPERNATE		COLLAGEN
			Mg. Lyophilized Material/Ml	Gly/Hypro	Mg. Lyophilized Material/Ml	Gly/Hypro	(Mg/Ml IN EXTRACT)
[2]K_2HPO_4-KH_2PO_4	1.5	7.6	0.84	7.2/3.3	4.9	3.5/0.5	0.2
[1]Na_2HPO_4-NaH_2PO_4	0.45	8.0	2.17	16.1/7.2	4.2	3.2/0.2	1.1
[2]Na_2HPO_4-NaH_2PO_4	0.45	7.6	1.42	15.2/7.35	5.5	3.4/0.3	0.74
[1]Na_2HPO_4-NaH_2PO_4	0.45	7.0	3.82	19.0/10.0	4.4	3.3/0.3	2.7
[4]Na_2HPO_4-NaH_2PO_4	0.19	5.9	2.74	9.2/3.9	3.76	3.2/0.4	0.76
[4]$NaC_2H_3O_2$-$HC_2H_3O_2$	0.45	4.0	2.4	15.3/7.0	6.9	17.8/8.4	5.3
[3]$NaC_2H_3O_2$-$HC_2H_3O_2$	0.19	4.0	4.22	20.4/10.9	2.78	7.1/2.7	3.8
[4]NaCl	2.57	Unbuffered	1.71	8.5/2.8	6.71	3.5/1.3	0.34
[3]NaCl	1.7	Unbuffered	2.2	12.5/5.1	7.23	3.5/0.3	0.8
[2]NaCl	1.5	Unbuffered	1.69	14.0/5.6	7.27	2.9/0.3	0.68
[2]NaCl	1.0	Unbuffered	2.73	15.6/7.7	8.1	3.6/0.3	1.5
[2]NaCl	0.7	Unbuffered	1.87	13.2/6.7	7.0	3.3/0.3	0.89
[2]NaCl	0.45	Unbuffered	1.27	14.0/6.1	7.3	3.3/0.3	0.55
[2]NaCl	0.2	Unbuffered	0.48	14.9/6.7	7.0	3.4/0.3	0.23
[1]Ox serum ultrafiltrate	..	7.4	1.05	10.7/5.8	5.1	3.5/10.2	0.42

* Extracts were dialyzed against water until salt-free. Precipitates and supernates were lyophilized, weighed, and analyzed for glycine and hydroxyproline. Collagen content of extracts was estimated from hydroxyproline values (based on 14 per cent for pure bovine corium collagen), and the amounts of material precipitated from the extract. Superscripts in first column indicate groups of extractions performed on the same sample of tissue.

In order to obtain information concerning the effect of temperature on the dissolved collagen, an aliquot of each extract was heated at 37° C. under toluene or filtered cyclohexane until a gelled opalescent precipitate formed. This was separated by centrifugation, resuspended and washed in fresh buffer in the centrifuge, dialyzed free of salt, and lyophilized. The degree of gelation and precipitation was determined every 15 minutes in all extracts by measuring turbidity in 2-ml. samples (held at 37° C.) in a photoelectric colorimeter.

RESULTS

Preliminary ultracentrifuge and electrophoretic studies were made on the phosphate and NaCl extracts. Extracts made with serum ultrafiltrate were examined by ultracentrifuge only. In all cases two or three slow-moving boundaries were present, together with at least one hypersharp peak. Four or five distinct components were observed electrophoretically. Attempts at separation and characterization of the components are in progress.

Ultraviolet absorption maxima occurred at 2600 A in a number of phosphate extracts, as reported earlier,[6] although this was by no means the rule, and, indeed, in some similar extracts maxima occurred at 2750–2800 A.

Collagen Content of Extracts.—White flocculent precipitates formed on dialysis against water in all cases except in the 1.0 and 1.5 *M* NaCl extracts, which set to stiff, slightly opalescent gels. The data concerning the lyophilized weights of the several salt-free fractions and their collagen content in terms of hydroxyproline and

glycine percentages are given in Table 1. Hydroxyproline content in the precipitates ranged from 4 to 11 per cent for all the salt extracts except at the highest ionic strength. Glycine was estimated relative to hydroxyproline in order to give some indication as to whether or not the collagen was abnormal with respect to hydroxyproline content. In all instances in which appreciable amounts of hydroxyproline were present, the ratio of glycine to hydroxyproline was about the same as that for purified bovine collagen. Thus the amount of hydroxyproline provides a good measure of the proportion of collagen in the various fractions. The hydroxyproline content of the lyophilized supernatant fluids were uniformly below 1 per cent except in the acetate extracts at pH 4, where they ranged as high as 8 per cent. Thus practically all the collagen in the neutral or near-neutral extracts was precipitated by dialysis against water. There was considerable variation in the amount of material extracted from different calf skins by the same extractant. For this reason the quantitative data in Table 1 are of comparative value only where they relate to simultaneous extractions of the same tissue samples by the different media. These groups are identified by superscripts.

The fact that collagen may be extracted from fresh native connective tissue by physiological media such as 0.2 *M* NaCl and bovine serum ultrafiltrate is in itself evidence that such soluble collagen could hardly have been derived from the formed fibrils of the corium.

Electron microscope examination of the precipitates revealed amorphous and thin filamentous material in some instances and in other cases large tangled masses of a long-spacing type of fibril displaying strongly accentuated double bands about 2000 A apart. This variability is as yet unexplained; it may relate to undetermined differences in composition between tissue samples. The precipitated collagen is completely soluble in the cold extraction media. However, much of the precipitated noncollagenous material remains insoluble.

In one experiment in which 400 gm. (dry weight) of calf corium were extracted four times with 10 per cent NaCl and 24 times with 0.15 *M* phosphate pH 8, a total of 38 gm., or 9.5 per cent of the total weight, was extracted. Soluble collagen (as estimated on the basis of hydroxyproline content, assuming 14 per cent for pure collagen) comprised 6.7 per cent of the material extracted and 0.6 per cent of the total corium. Even after 24 extractions the yield per extract was still appreciable although considerably diminished. Experiments of such long duration are complicated by bacterial growth, and one must reckon with the possbility of bacterial attack on the insoluble collagen.

Precipitation of Collagen from Neutral Salt Extracts by Heating.—It had been noted early that phosphate extracts which had been allowed to stand at room temperature for several days formed precipitates which, when examined in the electron microscope, proved to contain well-structured collagen fibrils. Heating these extracts at 37° C. greatly accelerated the precipitation. The extracts at pH 4 did not gel on warming, although fine amorphous precipitates formed.

Nearly all the collagen was precipitated, as indicated by the hydroxyproline and glycine content of the lyophilized precipitates and supernates (Table 2). The collagen thus precipitated from solution was quite pure, as was shown by the analyses and electron micrographs (Figs. 1 and 2).

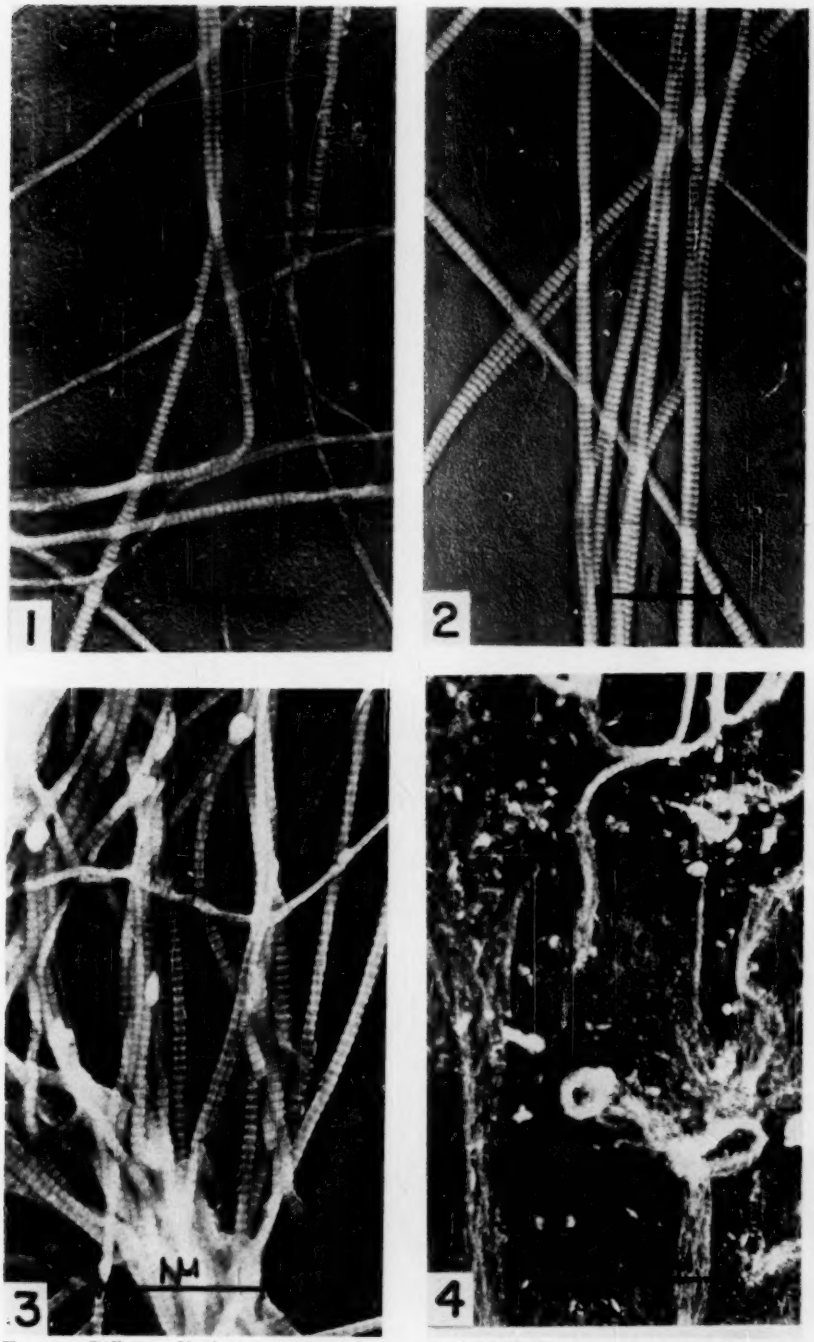

FIG. 1.—Collagen fibrils precipitated from a 0.45 *M* NaCl extract of calf corium by warming to 37° C. ×15,600.

FIG. 2.—Same as Figure 1, except that the source of collagen was the carp swim bladder tunic. × 16,700.

FIG. 3.—Collagen fibrils remaining after extraction of calf corium with 0.45 *M* NaCl. × 22,000.

FIG. 4.—Collagen remaining after extraction of calf corium with acetate buffer (pH 4.0, $\Gamma/2 = 0.45$). Note extensive disorganization of fibrils. × 18,000.

TABLE 2

PRECIPTATION OF COLLAGEN FROM CRUDE SALT EXTRACTS OF CALF CORIUM BY HEATING TO 37° C.

			Precipitate		Supernate	
Extractant	Γ/2	pH	Mg/Ml*	Gly/Hypro	Mg/Ml*	Gly/Hypro
Na_2HPO_4-NaH_2PO_4	0.45	8.0	0.80	26.4/13.6	6.0	4.2/1.0
Na_2HPO_4-NaH_2PO_4	0.45	7.0	2.12	27.1/13.1	6.1	4.6/1.1
NaCl	0.45	Unbuffered	1.25	24.7/13.1	6.3	4.1/0.1

* Lyophilized weight.

In the early experiments designed to study collagen which had been extracted by salt solutions, the collagen-containing fraction was further purified by dialyzing against water, lyophilizing the precipitate which contained nearly all the collagen, and re-extracting this material with fresh buffer. A considerable amount of the lyophilized material had become insoluble, but all the collagen could be dissolved out in four extractions. The extracts were extremely viscous and still heterodisperse, as was evident by the presence of more than one boundary in the ultracentrifuge. Such extracts, when heated to 37° C., formed stiff opaque white gels within 30 minutes. Electron microscope examination of the washed precipitates revealed typical collagen fibrils and some nonfibrous debris. Hydroxyproline and glycine determinations indicated that the precipitates consisted of fairly pure collagen. The sticky nature of the fibrous precipitates made washing and the preparation of electron microscope specimens difficult. Little hydroxyproline or glycine remained in the supernatant fluids.

Considerable differences were noted in the rate of gel formation on heating the different salt extracts of corium samples, even though they derived from the same animal. Extracts of ionic strength 0.45, 0.7, and 1.0[16] required about 6, 3, and $^1/_2$ hours, respectively, to gel.

The precipitation of collagen from crude salt extracts by heating is irreversible in the sense that cold salt solutions will not redissolve the precipitated collagen. However, weak acidic solutions will partially dissolve it, as they will also dissolve native collagen.

Among the salts tested no specific effects were noticed. Sodium salts of chloride, acetate, phosphate, and oxalate were about equally effective at the same ionic strength and pH. All the phenomena described above were reproduced with rat and guinea pig skin and carp swim bladder tunic.

Examination by electron microscopy of the tissue residues after salt extraction at neutral or near-neutral pH revealed normal unfrayed and unswollen collagen fibrils (Fig. 3). The residues remaining after extraction at pH 4 showed greatly swollen, frayed, and disorganized remnants of fibrils (Fig. 4).

DISCUSSION

It is evident that collagen can be extracted from connective tissues over a wide pH range. However, it is also apparent that the source and properties of the

extracted collagen differ not only with pH of the extraction medium but also with its ionic strength. On the acid side the great swelling and disorganization of the collagen fibrils is consistent with the view that the extracted collagen derives from these fibrils. At low ionic strengths, acid extracts of rat tail tendon or fish swim bladder tunic form clear gels of apparently structureless filaments after dialysis against water, whereas extracts made at the same pH but at higher ionic strength produce a mass of well-striated robust fibrils.

Extracts in the pH range between 5 and 9 differ significantly from those prepared at more acid pH. It is worth noting that this is the isoelectric range of collagen. The collagen fibrils in the residues appear to be unaltered, suggesting that the extracted collagen derived from extrafibrillar sources—either the ground substance or the cells. Practically all the collagen in these extracts may be precipitated in the form of striated fibrils by warming to 30°–37° C.; this is not true of acid extracts. The salt-extractable collagen most probably exists in a dispersed state in the tissue at this temperature. The fact that collagen extracted by cold neutral salt solutions is precipitated irreversibly at body temperature adds further support to the contention that this extractable collagen had not yet been incorporated into the formed fibrils.

It is interesting to speculate that the cell may secrete a precursor or precursors which, at least at one stage, are dispersed in the ground substance in the form of particles of subfibrillar dimensions, perhaps as "tropocollagen." The precipitation of fibrils may then proceed under the influence primarily of environmental factors such as ionic strength and temperature.

The high glycine turnover rate associated with the alkaline phosphate–extracted collagen, as compared to that of the other connective-tissue proteins,[9] strongly suggests a precursor role for this fraction. Whether or not there are metabolic, structural, or chemical differences in the collagen extracted at different ionic strengths remains to be determined.

When a gelatin solution is cooled, relatively larger aggregates are formed; these melt when the temperature is elevated to 28°–40° C.[17] Conversely, elevation of temperature of cold neutral salt solutions of collagen causes irreversible, specific aggregation of collagen chains in the form of cross-striated fibrils.

SUMMARY

Collagen may be extracted from whole intact connective tissues by serum ultrafiltrate and other neutral salt solutions. It is precipitated as typical cross-striated fibrils merely by elevating the temperature to 37° C. Such soluble collagen probably exists in the ground substance of the connective tissue rather than in the fully formed collagen fibrils of the corium. It is suggested that this collagen, possibly in the form of the "tropocollagen" monomer, is a precursor in physiological fibrogenesis.

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[5] M. B. McEwen and M. I. Pratt, in Randall, *op. cit.*, p. 158; P. Gallop, in press; S. E. Bresler *et al.* (*Repts. Acad. Sci. U.S.S.R.* [Moscow], **72**, 555, 1950) from ultracentrifuge and diffusion studies report a molecular weight of 70,000 for "procollagen." M. B. Mathews, E. Kulonen, and A. Dorfman (*Arch. Biochem. and Biophys.*, **52**, 247, 1954) report the same figure obtained by osmotic pressure studies on similar solutions. However, neither group determined whether or not the collagen in solution could be reconstituted to form fibrils, and there is reason to believe that in both cases the collagen was degraded to a "parent" gelatin.

[6] F. O. Schmitt, J. Gross, and J. H. Highberger, these PROCEEDINGS, **39**, 459, 1953.

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[10] J. H. Highberger, *J. Am. Leather Chemists Assoc.*, **9**, 345, 1936.

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TOPOLOGICAL PROPERTIES OF COILED HELICAL SYSTEMS

BY G. GAMOW

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Communicated November 12, 1954

In a recent article[1] Max Delbrück proposed a very ingenious way in which a dividing DNA molecule, constructed according to Watson and Crick's helical scheme,[2] can get disentangled and separated into two individual helices. The purpose of the present article is to indicate that there exists another, and in a way simpler and more natural, topological possibility of achieving the same result. In fact, a clean separation of two helices resulting from the division can take place without any breaks if, *prior to the division, the long helical molecule is wound into a coil possessing the same repetition period as the original helix.*

This fact can be easily understood by inspecting the coiling of nonhelical and helical two-stranded systems as shown in Figure 1, *A* and *B*. In the first case (*A*)

the "ribbon" formed by two strands is simply wound around the surface of a cylinder, and it is clear that the separation of the two strands without breaking or unwinding is quite impossible. In the second case (*B*) the twisting due to the original helical structure of the "ribbon" is compensated by the opposite winding due to coiling, and the separation of the two strands presents no difficulty.

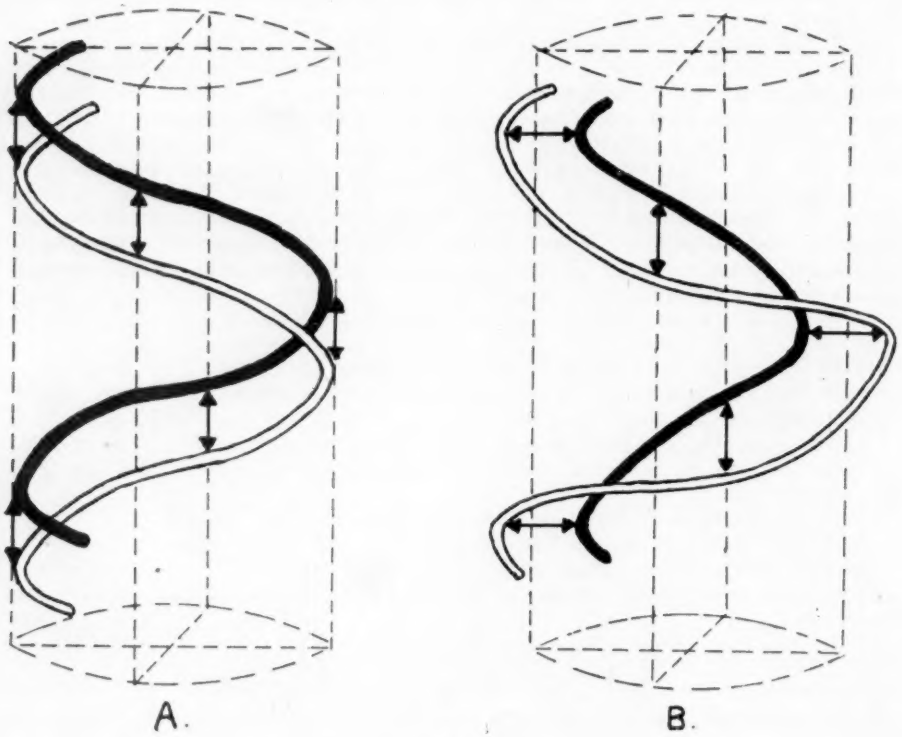

FIG. 1.

Since in the original Watson and Crick's model the repetition period of the helix is 43 A, the diameter of the coil should be somewhat less than $43/\pi = 13.7$ A. It is possible, however, that, owing to the change of intermolecular forces caused by coiling, the repetition period of the coiled helix becomes considerably larger than it was in the stretched-out state, thus leading to a coil of a much larger diameter.

A hypothesis may thus be suggested that the chromosomes, which become visible as comparatively thick rods in preparation for cell division, actually possess such a coiled helical structure. The coiling of the originally stretched-out helices becomes a necessary condition for the subsequent separation of the replicated molecular chains. In cases where, for some reason, the coiling does not take place and the replication occurs in th extended state, no separation of the replicated molecules is possible. This may be the explanation of the origin of the giant chromosomes in the salivary glands of *Drosophila melanogaster*.

It may be mentioned in conclusion that the topology of coiled helical structures may also throw some light on the properties of proteins which are biologically active in a globular (coiled) state and lose their specificity when denatured, i.e., transformed into a fibrous (uncoiled) state.

[1] M. Delbrück, these PROCEEDINGS, **40**, 783, 1954.
[2] T. Watson and F. Crick, *Nature*, **171**, 964, 1953.

THE ASSOCIATION OF IONS IN DIOXANE-WATER MIXTURES AT 25°

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Communicated November 10, 1954

In a recent paper Swarts and Kraus[1] have shown that, for many salts in aqueous solution, the conductance, as a function of concentration, may be expressed by the equation:

$$\Lambda = \Lambda_0 - (\Lambda_0\alpha + \beta)\sqrt{C} + BC. \quad (1)$$

Here Λ is the equivalent conductance at the concentration C, Λ_0 is the limiting equivalent conductance as C approaches zero, and α and β are constants whose values are fixed by the dielectric constant and viscosity of the solvent, the number of unit charges on each of the ions, and the absolute temperature. The coefficient B is an empirical constant which, for different salts, may be either positive or negative.

Onsager[2] has pointed out that, in deriving the simple equation

$$\Lambda = \Lambda_0 - (\Lambda_0\alpha + \beta)\sqrt{C}, \quad (2)$$

terms of order higher than $\sqrt{C}$, which appear in the expansion of the underlying functions, have been neglected. Most writers have assumed that B in equation 1 is necessarily positive for typical 1-1 electrolytes and that, when it is negative, it is indicative of ion association. Onsager has also pointed out that, when ion association occurs, the observed conductance will be less than is predicted by equation (2).

Many years ago, Fuoss and Kraus[3] measured the conductance of tetra*iso*amylammonium nitrate in dioxane-water mixtures over the complete range of composition from 0.0 to 100 per cent dioxane and evaluated the dissociation constant K of the ion-ion pair equilibrium for these mixtures. They found that the dissociation constant of the electrolyte increased with increasing dielectric constant in accord with Bjerrum's[4] theory. According to their results, $i\text{-Am}_4\text{NNO}_3$ is completely dissociated into its ions for mixtures having a dielectric constant greater than about 44. An examination of their early results shows that, in pure water, B has a negative value.

In the light of these considerations, it seemed worthwhile to make a careful investigation of several salts in dioxane-water mixtures from pure water to mixtures in which association occurs. Thus we have measured the conductance of solutions of tetra*iso*amylammonium nitrate, sodium bromate, and tetrabutylammonium

iodide and have determined the dissociation constant for these salts in the various mixtures. In general, as we have found, association does not set in until the dielectric constant reaches a value of about 50 or less. We have also measured the conductance of tetrabutylammonium bromide for mixtures containing 0.0, 10, and 20 per cent dioxane. As Swarts has shown, B is negative for $i\text{-Am}_4\text{NNO}_3$, Bu_4NI, and Bu_4NBr, while it is positive for NaBrO_3 in aqueous solution.

The techniques and procedures employed in the conductance measurements have already been adequately described in earlier papers.[5, 6] The conductance cell had a capacity of 1 liter of solution. All manipulations in which solvent or salt was introduced into the cell were carried out in a gloved box in which the carbon dioxide content of the atmosphere was controlled to a value where water, in equilibrium with this atmosphere, had a specific conductance of approximately 0.4×10^{-6}. The solvent content of the cell was determined by weighing on a sensitive balance.

Dioxane was introduced into the cell by means of a weight pipette having a capacity of approximately 300 cc. In place of a stopcock, the flow of liquid from the pipettes was controlled by means of a glass rod, located axially in the cylinder of the pipette, the end of which was ground to seat in the top of the delivery tube. This rod passed through a close-fitting tube at the top of the pipette and was held seated by means of a short length of soft rubber tubing. By raising the rod, the pipette could be emptied quickly and reproducibly. The concentration of dioxane in the mixture was known to 0.005 per cent.

In carrying out conductance measurements, the cell was placed in an oil bath whose temperature was controlled to 25° ± 0.001°, as indicated by a platinum resistance thermometer.

Dioxane was purified by the method of Hess and Frahm,[7] except that it was not recrystallized. All manipulations with dioxane were carried out under an atmosphere of nitrogen. The treated solvent was refluxed for 24 hours or more in the presence of sodium. It was distilled directly into the weight pipette.

In the early measurements the dioxane was not treated with hydrochloric acid. The conductance of solutions in mixtures with this dioxane exhibited a slow drift. This was determined, and the observed conductance was corrected in accordance with the observed drift. Subsequent measurements with stable solutions showed that the corrections were valid. The physical constants of the dioxane were found to be as follows: b.p., 101.3° at 760 mm.; m.p., 11.75°; sp. cond., $<1 \times 10^{-12}$.

All materials used in the preparation and purification of salts were carefully purified prior to use. Sodium bromate was recrystallized seven times from methanol-water mixtures. It was dried for 4 hours in a high vacuum at 70°. Tetrabutylammonium iodide (m.p., 147.7°–148.2°) was recrystallized six times from acetone and was dried for 48 hours in a high vacuum at 50°. Tetra*iso*amylammonium nitrate (m.p., 146.8°–147.3°) was recrystallized twice from ether-acetone mixtures and heated for 29 hours in a high vacuum. Tetrabutylammonium bromide (m.p., 119.2°–119.7°) was recrystallized four times from dry ethyl acetate. It was heated three days in a high vacuum at 50°. A metastable form of the salt melted at 104.3°–104.8°. All measurements for a given salt in different solvent mixtures were made with the same salt preparation.

TABLE 1

CONDUCTANCE OF Bu_4NI IN DIOXANE-WATER MIXTURES AT 25°

$C \times 10^4$	Λ	Λ_0'
A. 0 Per Cent Dioxane, D = 78.48		
1.06543	95.353	96.207
2.13809	94.999	96.207
4.25416	94.451	96.156
8.52156	93.627	96.039
17.0436	92.379	95.787
34.0819	90.537	95.351
68.6093	87.762	94.578
101.735	85.650	93.933
B. 15 Per Cent Dioxane, D = 66.10		
1.38044	69.827	70.659
2.75395	69.457	70.632
5.49901	68.908	70.568
10.9926	68.086	70.430
21.9703	66.862	70.173
43.8971	65.046	69.718
71.2758	63.296	69.237
98.6266	61.862	68.839
C. 30 Per Cent Dioxane, D = 53.28		
1.38095	53.009	53.779
2.75376	52.647	53.734
5.51113	52.127	53.664
10.9957	51.358	53.527
21.9843	50.225	53.288
43.9693	48.596	52.917
71.4135	47.071	52.565
98.8031	45.846	52.298

$C \times 10^4$	Λ	Λ_0'
D. 45 Per Cent Dioxane, D = 40.20		
0.55248	43.299	43.809
1.09870	43.025	43.744
2.19771	42.666	43.681
4.40496	42.170	43.606
8.80914	41.414	43.442
17.6263	40.321	43.182
35.2407	38.795	42.829
70.9958	36.717	42.421
E. 50 Per Cent Dioxane, D = 3[illegible].85		
0.85550	40.957	41.629
1.70600	40.611	41.559
3.40907	40.131	41.469
6.79380	39.417	41.303
13.5453	38.385	41.041
27.6291	36.892	40.671
55.7939	34.915	40.263
96.8348	32.983	40.009

TABLE 2

CONDUCTANCE OF $i\text{-Am}_4NNO_3$ IN DIOXANE-WATER MIXTURES AT 25°

$C \times 10^4$	Λ	Λ_0'
A. 0 Per Cent Dioxane, D = 78.48		
1.41151	88.318	89.281
2.83507	87.873	89.237
5.68994	87.253	89.187
11.4082	86.360	89.097
22.8073	85.068	88.935
37.6061	83.771	88.734
B. 10 Per Cent Dioxane, D = 70.33		
1.46436	73.933	74.820
2.92968	73.544	74.799
5.85455	72.986	74.759
11.6799	72.170	74.674
23.3327	70.991	74.528
46.5963	69.289	74.282
C. 20 Per Cent Dioxane, D = 61.86		
1.41159	62.304	63.111
2.83083	61.937	63.079
5.66830	61.428	63.043
11.3512	60.691	62.976
22.7511	59.623	62.856
34.9831	58.749	62.756
48.0602	57.959	62.652
61.6763	57.249	62.564
74.1693	56.665	62.492
88.3307	56.061	62.417

$C \times 10^4$	Λ	Λ_0'
D. 30 Per Cent Dioxane, D = 53.28		
1.44417	53.197	53.985
2.90278	52.866	53.984
5.81308	52.373	53.954
11.6316	51.660	53.896
23.3000	50.629	53.792
46.5964	49.187	53.656
E. 50 Per Cent Dioxane, D = 35.88		
1.43933	41.635	42.514
2.86712	41.220	42.460
5.75800	40.627	42.383
11.5401	39.798	42.281
23.0965	38.638	42.146
46.1817	37.092	42.047

TABLE 3

CONDUCTANCE OF $NaBrO_3$ IN DIOXANE-WATER MIXTURE AT 25°

$C \times 10^4$	Λ	Λ_0'
A. 0 Per Cent Dioxane, D = 78.48		
2.60004	104.465	105.833
5.20539	103.840	105.776
10.4396	103.071	105.813
20.8887	101.989	105.867
41.8148	100.508	105.998
83.6224	98.566	106.337
B. 10 Per Cent Dioxane, D = 70.33		
5.76328	88.525	90.385
11.4833	87.796	90.423
22.8762	86.764	90.472
45.6798	85.332	90.574
91.2034	83.466	90.880
C. 20 Per Cent Dioxane, D = 61.86		
2.79532	76.107	77.319
5.63373	75.561	77.282
11.2707	74.855	77.289
22.5158	73.873	77.313
45.0024	72.523	77.389
88.5707	70.794	77.628
D. 30 Per Cent Dioxane, D = 53.28		
5.82789	64.710	66.417
11.6555	63.986	66.399
23.3262	62.976	66.390
46.6973	61.594	66.425
93.3465	59.793	66.632
E. 35 Per Cent Dioxane, D = 48.91		
2.99942	60.529	61.760
6.05513	59.949	61.697
12.0914	59.182	61.653
24.1986	58.110	61.604
48.4159	56.656	61.598
96.9770	54.800	61.804
F. 40 Per Cent Dioxane, D = 44.54		
6.91601	55.637	57.540
13.8045	54.761	57.448
27.6820	53.567	57.370
41.4907	52.704	57.360
55.2810	52.001	57.376
110.736	49.984	57.604
G. 50 Per Cent Dioxane, D = 35.85		
5.89543	48.555	50.478
11.8047	47.566	50.282
23.5952	46.228	50.059
35.4006	45.259	49.946
47.2113	44.486	49.896
94.3569	42.304	49.958
H. 55 Per Cent Dioxane, D = 31.53		
5.93793	45.397	47.495
11.8609	44.247	47.202
23.6794	42.710	46.871
35.5217	41.609	46.696
47.3833	40.736	46.606
94.9003	38.329	46.638

TABLE 4

CONDUCTANCE OF Bu_4NBr IN DIOXANE-WATER MIXTURE AT 25°

$C \times 10^4$	Λ	Λ_0'
A. 0 Per Cent Dioxane, D = 78.48		
1.26249	96.564	97.496
2.53410	96.166	97.487
5.06128	95.623	97.489
10.1192	94.794	97.432
20.2585	93.570	97.302
B. 10 Per Cent Dioxane, D = 70.33		
1.33487	79.979	80.844
2.66113	79.604	80.826
5.29243	79.112	80.835
10.5890	78.355	80.792
21.2074	77.238	80.686
37.7717	75.963	80.563
C. 20 Per Cent Dioxane, D = 67.86		
1.36590	66.786	67.597
2.73642	66.471	67.618
5.47596	66.009	67.631
10.9592	65.310	67.605
21.8982	64.300	67.543
39.0061	63.128	67.455

The results of our measurements are presented in Tables 1–4, where C is the concentration in moles of solute per liter of solution and Λ is the equivalent conductance. The quantity Λ_0' in the third column is defined by equation (4) below; the quantity $\Lambda_0' - \Lambda_0$ is equal to the deviation of the observed value of Λ from that predicted by Onsager's simple equation (2). At the head of each table are given the composition of the solvent mixture and its dielectric constant, D.

In what follows, values of the dielectric constant of the mixtures have been interpolated from the data of Critchfield, Gibson, and Hall.[8] Viscosities have been interpolated from the data of Geddes,[9] after correcting to the latest values for the viscosity of water.[10]

If we are to evaluate the dissociation (or association) constant of an electrolyte in solution by the conductance method, we must know how the conductance of the free ions varies as a function of concentration. In computing the dissociation constant of electrolytes in solutions of lower dielectric constant, Fuoss and Kraus[3] and Fuoss[11] have assumed that the conductance of the ions conforms to Onsager's equation (2); in other words, they assume that there is no deviation from this equation within the concentration range under investigation. Shedlovsky[12] has computed dissociation constants on the assumption that the conductance of free ions deviates toward values of Λ greater than are predicted by equation (2) and that, moreover, this deviation is approximately a linear function of C. The recent investigation of Swarts[1] has shown that for many salts there is a linear deviation from Onsager's equation and that this deviation depends on the nature of the electrolyte and may be negative as well as positive. If we are to evaluate the dissociation constant of electrolytes in dioxane-water mixtures, we must find some means of determining the functional relation between Λ and C for the free ions.

Up to 0.01 N, the deviation, $\Delta\Lambda$, of the observed conductance from equation (2) is given within the 0.01 Λ-unit by the equations

$$\Delta\Lambda = \Lambda_0' - \Lambda_0, \tag{3}$$

where

$$\Lambda_0' \equiv \frac{\Lambda + \beta\sqrt{C}}{1 - \alpha\sqrt{C}}. \tag{4}$$

If Λ_0' is a linear function of C, as it is for many salts, we have

$$\Lambda_0' - \Lambda_0 = BC, \tag{5}$$

where B is an empirical constant which may be either positive or negative.

If we transpose the linear term, BC, of equation (1), we have

$$\Lambda - BC = \Lambda^* = \Lambda_0 - (\Lambda_0\alpha + \beta)\sqrt{C}. \tag{6}$$

The quantity Λ^* conforms to Onsager's equation and may be employed in evaluating the dissociation constant K by the method of Fuoss.[11]

In mixtures of dioxane and water the value of Λ_0 changes as a function of composition. In comparing the values of B in different mixtures, it is best to normalize equation (5) by dividing through by Λ_0. Thus we have

$$\frac{\Lambda_0'}{\Lambda_0} - 1 = B'C, \tag{7}$$

where

$$B' = \frac{B}{\Lambda_0}. \tag{8}$$

What we do is to evaluate B' for different mixtures in which ion association does not occur and plot values of B' against values of the dielectric constant, D. We find that B' is very nearly a linear function of D, and the plot may be extrapolated with considerable assurance to mixtures of lower values of D, where ion association occurs.

Knowing the value of B' and Λ_0, the value of B is known, values of BC may be computed to substitute in equation (6), and the value of Λ^* may thus be computed.

If ion association occurs, there will be a conductance decrease, $\Delta_a\Lambda$, due to ion association. If the decrease due to association is small, $\Delta_a\Lambda$ will approximate linearity, and we may write:

$$\Delta_a\Lambda = B_aC. \tag{9}$$

If this term is introduced into equation (6), we have

$$\Lambda_0' - \Lambda_0 = BC + B_aC = B^*C, \tag{10}$$

where

$$B^* = B + B_a. \tag{11}$$

Equation (7) then becomes

$$\frac{\Lambda_0'}{\Lambda_0} - 1 = B'^*C, \tag{12}$$

where

$$B'^* = \frac{B^*}{\Lambda_0}. \tag{13}$$

It is apparent that the limiting slopes of plots of Λ_0' or Λ_0'/Λ_0 versus C begin to increase (in a negative direction) when ion association sets in. However, the conductance change, $\Delta_a\Lambda$, due to ion association, is not employed explicitly in the evaluation of the association constant.

In Figure 1 are shown plots of Λ_0' versus C for Am_4NNO_3. The intercepts of these plots on the Λ_0' axis, on extrapolation, yield the values of Λ_0. The plots are linear at low concentrations up to about $2 \times 10^{-3}\,N$, where they exhibit an upward trend. For $D = 36$ this plot shows marked curvature at concentrations above $1 \times 10^{-3}\,N$. This is due to ion association.

In Figures 2 and 3 are shown plots of Λ_0'/Λ_0 versus C for Bu_4 NI and $NaBrO_3$, respectively, and in Figure 4 for Am_4NNO_3 and Bu_4NBr. The limiting slopes of these plots are determined by the values of B', or by B^* when ion association occurs. For Bu_4NI the slopes of all plots are negative and become increasingly so as the dielectric constant decreases. For $D = 40$ and $D = 36$, the slopes are very large, due to ion association.

For $NaBrO_3$ the slopes of the plots are initially positive but become negative for $D < 62$. There is a marked increase of the slope (negative) in going from $D = 53$ to $D = 49$, due to ion association. For $D = 32$ the slope has a large negative value due to the same cause.

For Am_4NNO_3 the Λ_0'/Λ_0 versus C plots coincide at lower concentrations for all values of D between 70 and 53, indicating that B' has the same value in all these

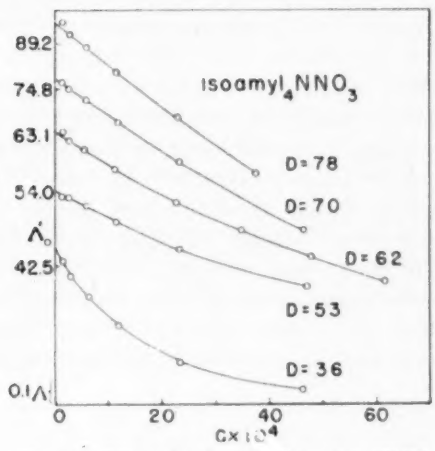

FIG. 1.—Plots of Λ_0' versus C for i-AmNNO$_3$ in different mixtures.

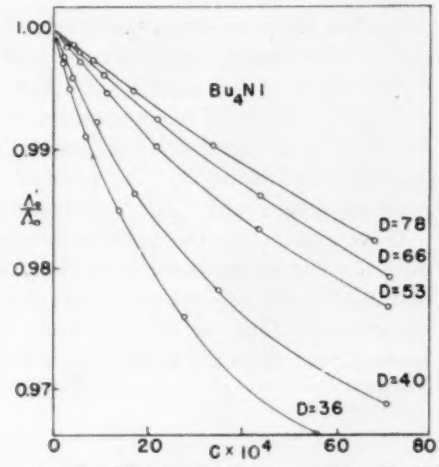

FIG. 2.—Plots of Λ_0'/Λ_0 versus C for Bu_4NI in different mixtures.

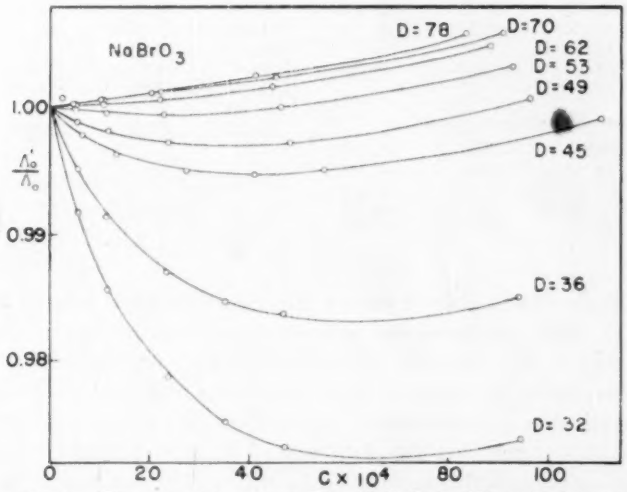

FIG. 3.—Plots of Λ_0'/Λ_0 versus C for $NaBrO_3$ in different mixtures.

mixtures. For $D = 36$ the plot has a large negative slope due to ion association. For Bu_4NBr the plots exhibit a slight trend toward lower slopes with increasing value of D.

In Table 5 are collected values of Λ_0, B, and B' for the four salts measured. An asterisk after a numeral indicates that the effect of ion association is included in the value of B or B'. It will be noted that there is a marked increase in the value of B or B' in going from unstarred to starred values.

The change in the value of B' due to ion association is best shown by plots of B', or B'^* versus D. In Figure 5 are shown plots of B' versus D for the four salts measured. For Bu_4NI and $NaBrO_3$ the plots are almost, but not quite, linear. For

Am_4NNO_3 the plot is linear and horizontal; for Bu_4NBr the plot has a slight upward slope. The broken lines of Figure 5 are not intended to show actual values of B' in the region of ion association. For $NaBrO_3$ it appears that the branch of the plot in the region of ion association runs tangentially into the branch at higher values of D, where there is no association. This is approximated by the plot for $NaBrO_3$. It follows that the value of D, where ion association sets in, cannot be precisely determined. For $NaBrO_3$, this lies between $D = 53$ and $D = 49$.

If we extrapolate the plots to mixtures of lower dielectric constant, we should obtain a fair approximation to the value of B' in these mixtures. Knowing B' and Λ_0, B may be computed and, therefore, the deviation BC from the Onsager equation and thus the value of Λ^* in equation (6). These values of Λ^* may then be employed to evaluate the dissociation constant K according to the method of Fuoss.[11]

TABLE 5

VALUES OF Λ_0, B, AND B' FOR SOME SALTS IN DIOXANE-WATER MIXTURES AT 25°

D	Λ_0	B	B'
A. Tetra-*n*-butylammonium Iodide			
78.48	96.27	−279	−2.9
66.10	70.70	−237	−3.4
53.28	53.81	−252	−4.7
40.20	43.78	−381*	−8.7*
35.85	41.67	−551*	−13.2*
B. Tetraisoamylammonium Nitrate			
78.48	89.27	−150	−1.68
70.33	74.84	−134	−1.79
61.86	63.11	−113	−1.79
53.28	54.01	−94	−1.74
35.85	42.55	−266*	−6.3*
C. Sodium Bromate			
78.48	105.75	59	0.56
70.33	90.37	48	0.53
61.86	77.27	21	0.27
53.28	66.43	−17	−0.26
48.91	61.76	−76*	−1.23*
44.54	57.66	−135*	−2.3*
35.85	50.72	−329*	−6.5*
31.53	47.89	−493*	−10.3*
D. Tetra-*n*-butylammonium Bromide			
78.48	97.56	−127	−1.30
70.33	80.89	−94	−1.16
61.86	67.66	−53	−0.78

In Figure 6 are shown Fuoss plots for $NaBrO_3$ in different solvent mixtures. In these plots we have employed the data at the concentrations of approximately 6×10^{-4} and 12×10^{-4}, since errors due to weighing are smaller here than at lower concentrations, while the concentrations are not so high that the Fuoss procedure becomes inapplicable. The values of $1/\Lambda_0$ derived from plots of Λ_0' versus C furnish a third independent point on the plots, since $F(z)/\Lambda = 1/\Lambda_0$ at $C = 0$. It will be noted that all points approximate linearity within 0.02 per cent. The plots serve well to illustrate the inherent difficulties underlying the precise determination of the association constant for electrolytes when the degree of association of their ions is very small.

Values of the dissociation constant, K, as so determined, are shown in the third column of Table 6. In the fourth column are shown values of K_F computed on the assumption that the conductance of the ions conforms to Onsager's equation (2). Values of B employed in the computations are shown in the fifth column and values of the dielectric constant, D, in the second column.

As was to be expected, K is greater than K_F, since we have found a negative deviation from equation (2) in all cases. With decreasing dielectric constant, the value of K decreases approximately in accordance with Bjerrum's theory. A precise comparison with theory cannot be made because the degree of association is so low that errors in the value of K are large, particularly when K itself is large.

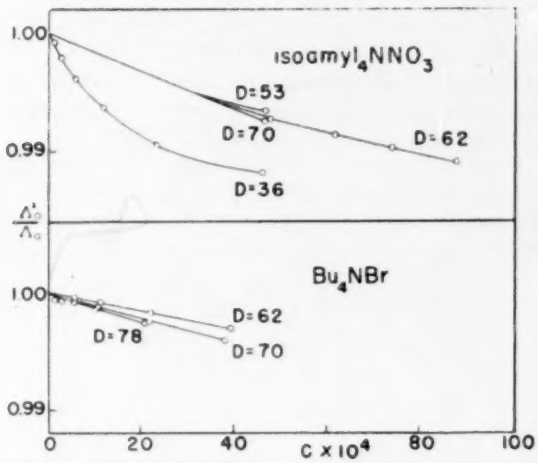

FIG. 4 (right).—Plots of Λ_0'/Λ_0 versus C for i-Am$_4$NNO$_3$ and Bu$_4$NBr in different mixtures.

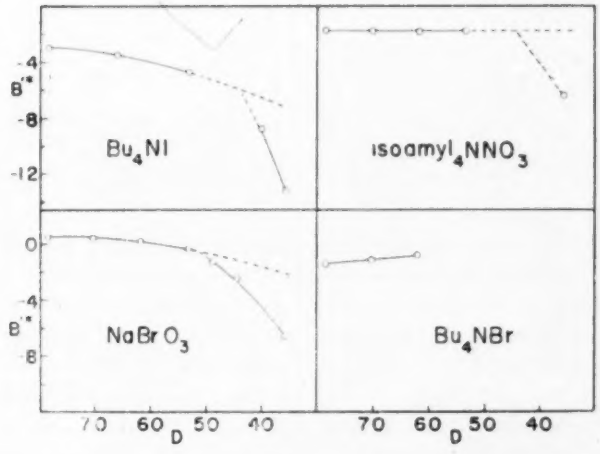

FIG. 5 (left).—Plots of B' versus D for salts in different mixtures.

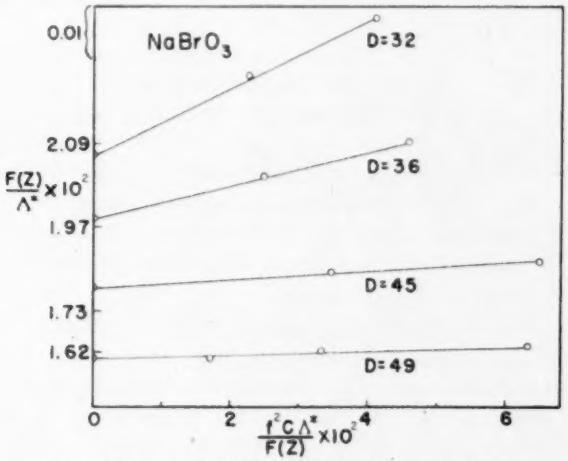

FIG. 6.—Fuoss plots for $NaBrO_3$ in different mixtures.

To illustrate this point, we give some numerical values of quantities for $NaBrO_3$ in Table 7. The deviation, BC, from equation (2) is given in the third column; the conductance decrease, $\Delta_d\Lambda$, due to ion association is given in the fourth column; the percentage of association, $100(1-\gamma)$, in the fifth column; and the activity coefficient, f, in the sixth column.

TABLE 6

VALUES OF DISSOCIATION CONSTANTS, K AND K_F, FOR SOME SALTS IN DIOXANE-WATER MIXTURES AT 25°

Salt	D	K	K_F	B
Bu_4NI	40.20	0.36	0.095	−285
Bu_4NI	35.85	.126	.060	−304
Am_4NNO_3	35.85	.113	.092	−77
$NaBrO_3$	48.91	.88	.52	−37
$NaBrO_3$	44.54	.43	.28	−52
$NaBrO_3$	35.85	.130	.098	−96
$NaBrO_3$	31.55	0.072	0.054	−145

TABLE 7

DEGREE OF ASSOCIATION OF $NaBrO_3$ IN DIOXANE-WATER MIXTURES

D	$C \times 10^4$	BC	$\Delta_d\Lambda$	$100(1-\gamma)$	f
48.91	6.05513	−0.022	−0.038	0.08	0.943
48.91	12.0914	.045	.066	0.11	.920
44.54	6.91601	.036	.084	0.15	.930
44.54	13.8045	.072	.140	0.25	.903
35.35	5.89543	.057	.183	0.38	.912
35.35	11.8047	.113	.327	0.68	.878
31.53	5.93793	.086	.304	0.68	.894
31.53	11.8609	0.172	0.518	1.15	0.854

It will be noted that for the most concentrated solution in the mixture of lowest dielectric constant ($D = 31.53$) the conductance change due to ion association is only 0.518 Λ-unit, and the degree of association is only 1.15 per cent. For the other solution these quantities are much smaller. For $D = 48.9$ and $C = 6.06 \times 10^{-4}$, $\Delta_d\Lambda = 0.038$, and the association is 0.08 per cent. In the mixtures of lowest dielectric constant, the value of K might be in error by as much as ±2 per cent, and in the mixture of $D = 49$ by as much as ±20 per cent.

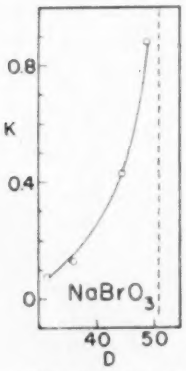

FIG. 7.—Plot of K versus D for $NaBrO_3$.

In Figure 7 is shown a plot of K versus D. As may be seen, as D increases, K increases greatly and approaches an asymptote in the neighborhood of $D = 52$. Qualitatively, our results are in accord with Bjerrum's theory. There can be no doubt that these salts are completely dissociated in mixtures having dielectric constants greater than about 50. The method which we have employed to determine the deviation from Onsager's equation in these mixtures enables us to evaluate K with a fair degree of approximation. It is apparent that the evaluation of K in pure solvents of high dielectric constant is subject to rather large uncertainty.

The viscosity of dioxane-water mixtures increases greatly as their dioxane content increases, reaching a maximum 2.27 times that of water at 61.5 per cent dioxane. As a result of increasing viscosity, the limiting conductance, Λ_0, decreases greatly for all salts. However, the effect of viscosity on conductance differs markedly for different salts.

According to Walden's rule,[12] the conductance-viscosity product, $\Lambda_0\eta$, should have a constant value for a given electrolyte in solvents of different viscosities. While this relation holds as a rough approximation, the manner in which the product changes for different salts in the same solvent mixtures varies greatly.

In Figure 8 are shown plots of $\Lambda_0\eta$ for Am_4NNO_3, Bu_4NI, Bu_4NBr, and $NaBrO_3$. It will be noted that the plots for these salts differ greatly. For $NaBrO_3$ the product passes through a pronounced maximum at about 25 per cent dioxane and returns to near its value for water at 55 per cent dioxane. For Bu_4NI the product falls off continuously with increasing dioxane constant; it shows signs of leveling off at 50

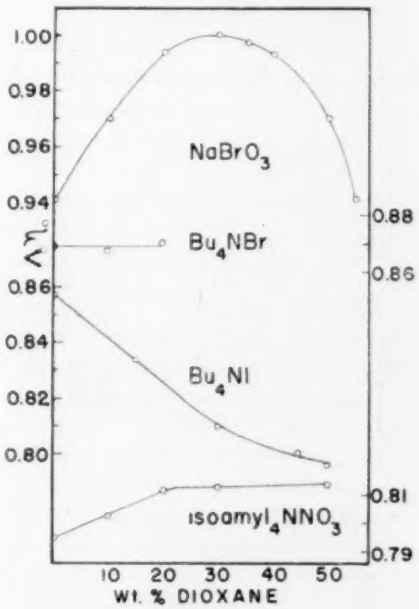

FIG. 8.—Plots of $\Lambda_0\eta$ product for salts in different mixtures.

per cent dioxane. For Am_4NNO_3 the product increases slightly up to 20 per cent dioxane and thereafter does not change up to 50 per cent dioxane. The product for Bu_4NBr shows little change up to 20 per cent dioxane. Since the bromide and the iodide have the same cation, the effect of viscosity on the conductance of bromide and iodide ions differs greatly.

These results illustrate the striking fact that the interactions of the ions with solvent molecules, which in the final analysis determine the mobility of the ions, are highly specific. Further studies with solvent mixtures might well serve to shed some light on the presently obscure problem of ion mobilities.

* The investigation here reported was supported by the Office of Naval Research under contract N7onr-35809. This paper is based on a portion of a thesis presented by Robert W. Martel in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Brown University, June, 1954.

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*A TEST FOR RECOMBINATIONAL LETHALS IN THE X-CHROMOSOME OF DROSOPHILA MELANOGASTER**

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Communicated by Curt Stern, November 1, 1954

In recent years it has been demonstrated that, when two chromosomes of normal but different stocks have been allowed to recombine, the resultant chromosomes, when made homozygous, may exhibit an array of viabilities totally unlike the homozygotes of the original chromosomes. In addition, the recombinants may prove to carry lethals even though the original chromosome carried none.

Dobzhansky,[1] in 1946, showed what an array of viabilities could be produced by recombination within the second chromosome of *Drosophila pseudoobscura*. For example, from a heterozygote for two nonlethal second chromosomes, thirty-three chromosomes were extracted and tested for viability. These would consist of noncrossovers and various recombinations of the two original types. The expected frequency of wild-type flies was 33.3 per cent, yet frequencies ranging from 5.7 to 38.4 per cent were found. In two other tests involving a chromosome that was originally lethal, Dobzhansky found further lethals which he ascribed to recombination of the original chromosomes. Misro,[2] in 1949, studied the same phenomenon in the second chromosome of *Drosophila melanogaster*, using normal chromosomes from a wild population. The difference between the higher number of lethals obtained from heterozygous females as compared to lethals from homozygous females and heterozygous males was ascribed to crossing-over and recombination between the normal chromosomes. Of the twenty-five separately arising lethals from heterozygous females, none were found to be allelomorphic, which indicated to Misro that the amount of variation in regard to a single character was very great. Wallace,[3] in 1953, obtained results similar to those of the above authors from experiments with fifty-five combinations of ten different second chromosomes extracted from two experimental populations of *D. melanogaster*. Since recombination may produce such drastic results in the second chromosome, it would be of interest to learn whether recombination in the X-chromosome has the same effect. The work described here was done to learn whether crossing-over and recombination between

normal X-chromosomes from different wild-type populations of *D. melanogaster* which have no lethal or strikingly detrimental viability effects would result in the production of lethals.

Material and Methods.—Ten different wild-type laboratory stocks were used. One of the stocks came from Formosa and one from Argentina, while the rest were selected at random from widely divergent localities within the United States. All had been kept as laboratory stocks in the Zoölogy Department and in the Genetics Division at the University of California at Berkeley for at least three years when this series of experiments was started in 1953. One male was taken from each of the ten stocks and mated with *y f* attached-X females. The offspring were composed of two classes: wild-type males with their X-chromosomes derived from the male parent and *y f* females with their X-chromosomes both derived from the parent females. The experimental wild-type X-chromosomes of the ten types were perpetuated at 26° C. in further matings with their attached-X sibs, and thus there was chance neither for lethals to accumulate nor for crossing-over. Examination of salivary glands of the wild-type flies showed no inversions in the X-chromosomes used.

In the experimental crosses it was necessary to employ a crossover suppressor chromosome. For this purpose an inversion chromosome with the markers $y\ sc^{S1}\ B$ In 49 $v\ w^a\ sc^8$ was employed in one step, and another, designated "Binscy" and having the markers $y\ sc^{S1}\ B\ In\ 49\ sc^8$, in a later step. Both of these stocks originally came from Dr. H. J. Muller's laboratory. A sample procedure of the crosses is outlined below ("Cor" stands for a wild-type stock collected at Corona, California, and "Sal" is a wild type collected at Salta, Argentina):

G1: 1 $y\ B\ v\ w^a$/Cor ♀ × 3 or 4 Sal ♂♂
G2: 4(1 Cor/Sal ♀ × 4 Binscy ♂♂)
G3: 25(1 Cor-Sal/*B* ♀ × 4 Binscy ♂♂)
Possible offspring of G3: Cor-Sal/*B* ♀♀; *B*/*B* ♀♀; *B* ♂♂; + ♂♂

G1: One $yBvw^a$/Cor female was crossed with three or four Sal males. More than one male was used to minimize the chance of encountering lethals from gonial clusters.

G2: Four Cor/Sal virgin females (Roman numerals I through IV in Table 1) were taken from the offspring of G1 and mated singly to four Binscy males.

G3: Approximately twenty-five *B*/+ virgin female offspring (Cor-Sal/*B* ♀) from each of the four Cor/Sal females were mated separately to four Binscy males. Fifteen days later the offspring were checked for lethals carried by the G3 motners. These *B*/+ females of G3 may have had an X-chromosome that was composed entirely of the Salta X-chromosome or entirely of the Corona X-chromosome, or it may have been composed of various combinations of the two chromosomes. If no lethal was present in the + chromosome, then males with round red eyes (+) were expected. A creamer was designated as lethal if it contained at least twenty flies but no + males. If there were less than twenty flies and no + males, the creamer was designated as doubtful. In either case, *B*/+ female offspring whose + chromosomes were identical with the supposedly lethal or doubtful + chromosome of their nonappearing male sibs were mated with Binscy males, and 15 days later their progeny were checked for verification of the earlier findings. In this

test the presence of fifty flies without any + males was regarded as the minimum criterion for lethality. Where, for G3, twenty-five $B/+$ females could not be obtained from one $+/+$ mother (G2), more were collected from others of the four in order that approximately a hundred chromosomes could be tested. In Table 1 numerals V–VIII indicate a repeat test.

TABLE 1

FREQUENCY OF LETHALS*

Class	I	II	III	IV	V	VI	VII	VIII	Total
				Homozygotes					
Tem/Tem	0/7	0/9	0/9	0/9	...	...	...	...	0/34
Sal/Sal	0/25	0/25	3/25†	9/25†	0/26	0/26	0/34	0/30	0/166
For/For	0/25	0/28	1/20	0/27	...	...	...	...	1/100
Total for other 7 homozygous classes‡	...	...	...	...	...	...	...	...	0/694
Total	...	...	...	...	...	...	...	...	1/994
				Heterozygotes					
Tem/Sam	1/23	0/30	0/29	0/18	...	...	...	...	1/100
Sam/Ore	0/22	2/25	0/22	0/27	...	...	...	...	2/96
Sam/Can	1/24	0/25	0/25	0/24	...	...	...	...	1/98
Fla/Sac	0/25	0/24	1/25	0/25	...	...	...	...	1/99
Fla/Cor	2/24	0/25	0/25	0/23	...	...	...	...	2/97
Fla/Sal	0/25	0/25	1/25	0/25	0/42	0/26	0/28	0/33	1/229
Sac/Sal	0/25	0/24	11/24†	0/24	0/23	0/52	0/51	0/68	0/267
Fre/Sal	0/25	0/25	1/24	1/25	0/33	0/30	0/54	0/38	2/254
Cor/Sal	0/23	1/26	0/25	0/25	0/34	0/31	0/42	0/28	1/234
Sal/Ore	0/30	1/31	0/30	0/9	0/31	1/69	0/18	0/28	2/246
Total for other 35 heterozygous classes‡	...	...	...	...	...	...	...	...	0/3466
Total	...	...	...	...	...	...	...	...	13/5186

* Classes indicate combinations of wild-type X-chromosomes. Roman numerals indicate F_1 females (G2). I through IV within each class are from a single G1 culture. V through VIII represent a repeat test under similar conditions. In the fractions the numerator indicates the number of lethals found and the denominator the number of chromosomes tested.

† Females probably carry pre-recombinational lethals and therefore are not included in the totals or statistical analysis.

‡ Explained in text.

A possible source of lethals would be mutations occurring before generation G2. If such a pre-recombinational lethal were included in a culture, the Cor/Sal female of G2 would be heterozygous for the lethal. It would be detected because of several lethals appearing among the offspring of one mother. These lethals would be allelic and in the ideal case would include 50 per cent of the chromosomes tested.

Since 10 wild-type chromosomes were used, there were 55 possible combinations. Of these, 10 were homozygous for each wild-type X-chromosome and served as controls. The other 45 combinations were heterozygous for the X-chromosomes. It is from these heterozygotes that one would expect an increase in the frequency of lethals if recombination between the X-chromosomes were responsible for their production. Eight generations elapsed between the first and the last test, and seven more generations elapsed before the repeat test.

Results.—The results are presented in Table 1. Seven homozygous classes which had no lethals are not individually tabulated, nor are the thirty-five heterozygous combinations without lethals. The range in chromosomes tested in each homozygous class not individually tabulated was 98–100, with four F_1 mothers (G2) tested within each class. Twenty-two mothers had a range of from 22–30 chromosomes tested, two each had 32, one 31, one 19, one 18, and one had 6 tested. Tem/

Tem has low values because sixty-six cultures were killed accidentally by heat. For the thirty-five heterozygous classes not individually tabulated, the range in chromosomes tested was 96–100, with four F_1 mothers (G2) being tested within each class. One hundred and twenty-six mothers had a range of from 20 to 30 chromosomes tested; one each had 37, 32, 17, 16, 14, 11; three each had 31, and 3 each had 19; while two had 15 chromosomes tested.

Only one lethal (0.10 per cent) was found in 994 homozygous controls (excluding those which were of possible pre-recombinational origin). Thirteen (0.25 per cent) were found in 5,186 chromosomes tested from the heterozygous class (excluding one which presumably was pre-recombinational). Of these thirteen, probably only twelve were of separate origin, since on one occasion (Sam/Ore) two lethals arose from one mother and were probably derived from a gonial cluster. The two lethals, respectively, gave 0 or 1 crossover with *forked* among more than three hundred flies each. They are probably at the same locus. In a second case (Fla/Cor) two lethals arose from one mother, but crossover tests place the lethals at different loci. One was found to be at the *yellow* locus, and the other was between *cv* and *v*. Approximately three hundred flies were examined in each test. In two instances two lethals arose within the same class but from two mothers. These were independent lethals.

In the statistical analysis of the significance of the difference between the numbers of control and experimental lethals, the value 13 for the experimentals was used to give the benefit of the doubt to the hypothesis that lethals are produced by recombination of two normal X-chromosomes. The Brandt-Snedecor Test gave a probability of 0.3, indicating that chance alone would account for the difference in production of lethals as exhibited between the homozygous and the heterozygous classes. In one control case (Sal/Sal = $^3/_{25}$) the lethals may have resulted from a gonial mutation producing three lethals from one mother. If this is true, then one more lethal should be added to the control, whose rate would then be 0.19 as opposed to 0.25 per cent for the experimentals.

Genetical localization tests of the lethals, using the markers *y cv v f*, are compatible with the assumption that all the lethals from these heterozygotes have specific loci and are not due to recombination. Without the knowledge of the aforementioned results on the second chromosome, there would be little reason to suspect that recombination had played any part in the production of lethals in the X-chromosome. Even if lethals should be produced in the X-chromosome by recombination, the frequency of this event would be low. Since the X-chromosome, which is carried in the hemizygous condition in the male, is under very high selection pressure, it is perhaps not surprising that recombination has such little effect.

Summary.—This experiment indicates that statistically there is no reason to assume that recombination plays a significant role in producing lethals in the X-chromosome. The specific localization of lethals supports the interpretation that they did not originate by recombination. These results differ from those of other authors on the second chromosome, where the high production of lethals is considered to be caused by recombination.

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WAVES WITH Pn AND Sn VELOCITY AT GREAT DISTANCES

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Since the completion of installation of the long-period three-component seismograph ($T_0 = 15$ seconds, $T_g = 75$ seconds) at Palisades in June, 1953, seismograms have revealed new phases and have given new information about old ones. In this paper we report observations of phases related to Pn at epicentral distances of 69°–79° and to Sn at distances of 52°–125°. At such distances these phases must result from wave-guide action in which compressional waves in one case and shear waves in the other are trapped beneath the Mohorovičić discontinuity. As in all wave-guide phenomena, these phases may be considered as interference patterns resulting from superposition of many pulses, each of which may be identified by its path through the wave guide.

In Table 1 the observational data for these new phases are summarized. The list represents typical occurrences at Palisades and is not a complete tabulation. Many repetitions of the shocks confirm the readings reported, often in astonishing detail.

TABLE 1

No.	Date	Origin Time	Lat.	Long.	Azimuth	Mag.	Depth (Km.)	Δ	Sn Vel. (Km/Sec)	Pn Vel. (Km/Sec)
1	25 Aug. 1953	02:04:16	4½° S.	152¾° E.	299°	6½–6¾	.	125.4°	4.57	....
2	22 July 1953	05:11:15	51° N.	157° E.	330°	6¾–7	60	78.2°	4.62	8.24
3	9 Aug. 1953	07:41:06	35½° N.	21° E.	57°	6¾	...	69.3°	4.59	7.98
4*	13 Aug. 1953	09:23:33	21½° S.	170° E.	269°	6¾–7	150	123.3°	4.42	....
5	4 Sept. 1953	07:23:05	51° N.	156¼° E.	331°	6¾–7	60	78.5°	4.61	8.18
6*	2 July 1953	06:56:55	19° S.	168¼° E.	272°	7¾	200	123.1°	4.36	....
7	15 June 1953	17:47:14	56½° N.	154° W.	316°	6½	...	52.0°	4.63	....
8†	26 Feb. 1953	11:42:30	11° S.	164¼° E.	284°	7¼	...	121.1°	4.44	....
9	25 Feb. 1953	21:16:18	56° N.	156½° W.	316°	6¾	60	53.5°	4.57	....
10	12 Feb. 1953	08:15:32	35.8° N.	55° E.	39°	7	...	90.5°	4.52	....
11	29 July 1953	23:18:02	16° S.	173° W.	264°	6½	...	107.1°	4.59	....
12	26 July 1953	16:53:18	17.8° N.	145.2° E.	320°	7	200	111.2°	4.50	....
13	30 Apr. 1954	13:02:36	39.5° N.	22.2° E.	56°	7	...	69.4°	4.56	....
14†	21 June 1954	01:48:44	23° S.	68½° W.	174°	6½–6¾	150	63.9°	4.34	....
15†	30 July 1954	08:46:00	36½° S.	97° W.	199°	6½	...	80.0°	4.42	....

* Questionable. † Sn—Type II.

Waves with Pn Velocity.—A typical record of these waves is presented in Figure 1. The new phase is best seen on the vertical seismogram as a conspicuous train of oscillations, with a dominant period of 12 seconds, ranging down to 8 seconds, duration of 80 seconds, and amplitude approximately equal to that of PP, arriving shortly after PPP but easily distinguished from it by virtue of a shorter period.

Accumulation of data on this phase is somewhat slow because it is only in the range 70°–100° that Pn is free of interference from PPP and PcS on the one hand and SKS and later body phases on the other. Even within this range the residual motion is often so large as to obscure the Pn wave group.

The velocity of the phase, determined for the beginning motion, ranges from 7.98 to 8.24 km/sec, a value in excellent agreement with recent results from studies of explosions, rockbursts, and near-earthquakes.

Waves with Sn Velocity.—This phase occurs with several times the frequency of the corresponding Pn arrival. Two types appear to exist. The first (Figs. 1 and 2) is characterized by large vertical motion consisting of one or two oscillations with

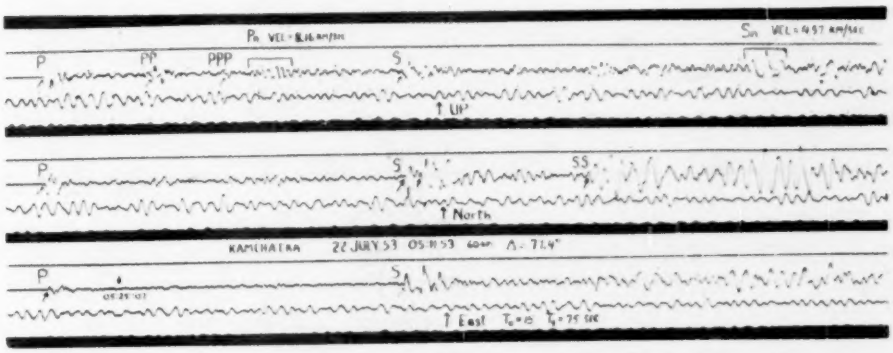

FIG. 1.—Phases with Pn and Sn velocity for earthquake No. 2

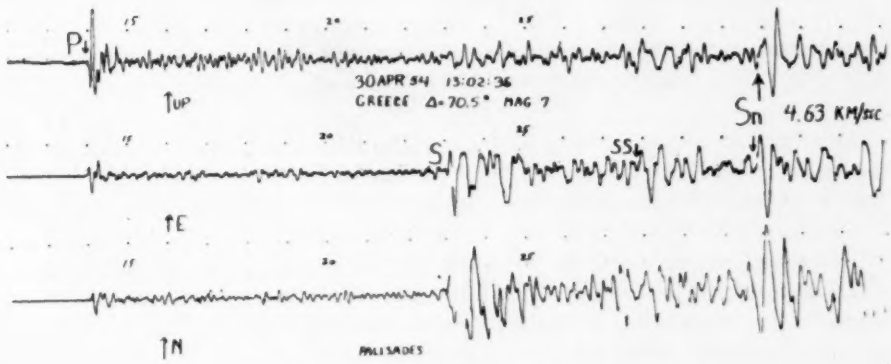

FIG. 2.—Phase with Sn velocity for earthquake No. 13

periods of about 20–30 seconds. Often there is large motion in the radial horizontal direction at the same time but incoherent with respect to the vertical motion. The average velocity of the phase is about 4.58 km/sec.

The second type appears to be associated with the long-period waves ("G") which initiate the dispersive train of Love waves. Motion on the transverse com-

ponent consists of an impulsive initiation of G. Ordinarily one would not distinguish this as a separate phase, but very often this impulsive movement is accompanied by an arrival on the vertical seismogram (Fig. 3). Too few data are available for a firm conclusion, but there is a suggestion that the second type travels with a somewhat slower velocity of about 4.40 km/sec and occurs principally for long oceanic paths.

Discussion.—Professor Gutenberg has brought to our attention a recent paper by Caloi[1] in which similar phases are described. Caloi finds velocities of 7.9–8.0 km/sec for the Pn type and 4.4 km/sec for the first Sn type. He reports that the waves are best excited when the earthquake focus lies near the low-velocity channel in the mantle. He does not discuss the second Sn type.

Caloi suggests that these phases are propagated in the wave guide formed by Gutenberg's[2] low-velocity channel in a manner analogous to Sofar propagation[3] in the oceans. The fact that his observed velocities are somewhat lower than the velocity of Pn and Sn observed in explosion seismology studies seems to support this conclusion.

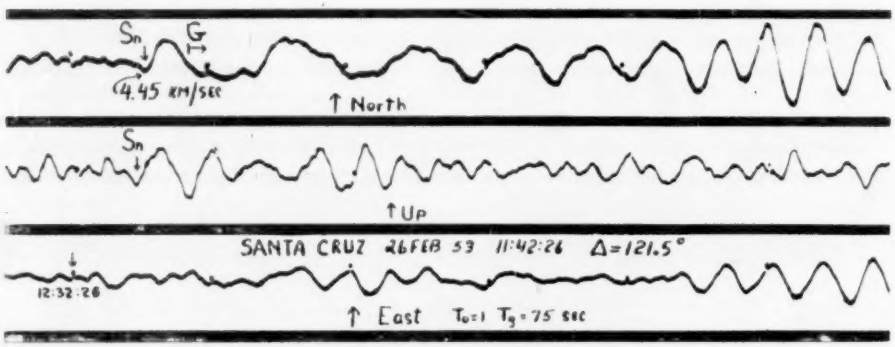

FIG. 3.—Shear phase (Type II) for earthquake No. 8

As may be seen from Table 1, our data, on the other hand, do not as yet show any obvious relation between focal depth and excitation. Moreover, our velocities for the Pn type and Sn type of the first kind approach the values observed for Pn and Sn in explosion seismology studies. These facts suggest an alternative mechanism of transmission to us, involving "whispering gallery" propagation in the mantle by multiple grazing reflection from the Mohorovičić discontinuity. Rayleigh[4] (**2**, 128) discussed the whispering gallery many years ago and suggested that this type of propagation might occur within the earth. This mechanism may be likened to propagation in a wave guide consisting of a half-space in which the velocity increases with depth. In Fig. 4 we have sketched diagrammatically the two types of propagation suggested for these phases.

Additional data are needed before one can decide between the two modes of propagation. Clearly, theoretical study is necessary to ascertain whether sufficient energy can be propagated along these paths when sources and detectors are displaced from the actual channel. The effect of the crust and the great difference in character between the Pn and Sn type which may be seen in Figure 1 must be

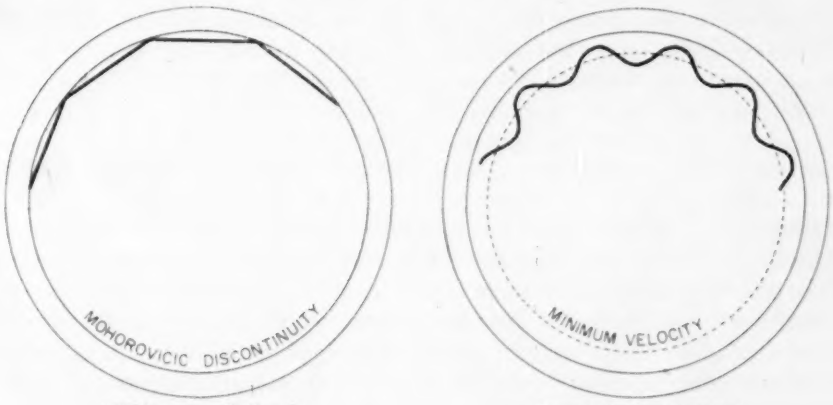

FIG. 4.—Diagrammatic representation of two mechanisms for long-distance propagation of compressional and shear phases in upper mantle.

accounted for. Until this is done, the mode of propagation must be considered uncertain.

Whatever the mechanism of propagation, the new phases have great significance. They may be used to deduce the velocity in the channel through which they travel and to test whether there are differences in structure and composition of the upper mantle beneath continents and oceans.

Summary.—Earthquake phases which propagate to large distances with velocities close to those observed for Pn and Sn in explosion and near-earthquake studies are reported and described. A mechanism of transmission involving "whispering gallery" propagation in the mantle by multiple grazing reflections from the Mohorovičić discontinuity is tentatively suggested.

[6] This is Contribution No. 131 of the Lamont Geological Observatory of Columbia University.

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ON THE ORIGIN OF TEKTITES

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Communicated November 19, 1954

The terrestrial or extraterrestrial origin of tektites has been a subject of controversy for years.[1] These glassy objects have been found in localized areas of the earth having linear dimensions of the order of thousands of kilometers. About ten groups are distinguished on the basis of locality, chemical composition, and detailed physical properties. Each of these groups of objects has fairly constant chemical

composition, and the groups differ from one another in composition. Since each group is scattered over a wide area and over many different kinds of rocky formations, it seems that these tektites cannot be derived from the local rocks. Their chemical composition is remarkably similar to argillaceous sediments, and probably some rather common sedimentary material can be found which on fusion would duplicate the composition of each group. This is the most potent argument for a terrestrial origin.

The most important arguments for an extraterrestrial origin are the following: (1) The melting point of tektites are so high that no known terrestrial temperature is adequate for melting them except lightning, which, however, is not observed to produce objects resembling tektites when it does strike the earth. (2) No volcanoes are distributed in such a way as to produce their observed distribution, and the temperatures associated with volcanoes are not sufficient to melt them in any case. Hence, by elimination, it is argued that they are a form of meteorite. The author has weighted these arguments in favor of the extraterrestrial origin and has discussed with colleagues and students at the University of Chicago certain ideas in regard to such an origin.

The Moon as the Origin of Tektites.—That tektites were objects thrown off from the moon was suggested by Verebek in 1897. Barnes[1] cites the literature on this subject and discusses it, though I am not convinced by his arguments that this is not a possible source. Many swarms of tektites may have arrived, instead of only one as he suggests, and they need not have fallen on one great circle of the earth, as proponents of the idea have suggested and as he shows to be untrue.

If tektites are extraterrestrial, their distributions on the earth put definite limitations on their origin. As stated above, each group is distributed over large areas with linear dimensions of some thousands of kilometers; e.g., the australites are distributed over southern Australia and Tasmania. This means that they could not have arrived as one object which broke up in the atmosphere, for in that case such a wide distribution would be impossible. They therefore arrived as a swarm of objects. These could not have been moving in circumsolar orbits, for, unless the swarm had a density of 10^{-6} gm/cm^3 or more, it would have been scattered by the sun's gravitational field. But, if it had this density and the dimensions of these tektite areas, the tektites would fall with a surface density of around 100 gm/cm^2 (i.e., 10^{-6} gm. cm.$^{-3}$ times 10^8 cm.), which is definitely not observed. Hence their source must have been nearer, and perhaps they may have arrived from the moon. Perhaps some initial conditions for departure from the moon would permit arrival on the earth in localized areas as observed. One is surprised that they do not become scattered more widely than they are, but we assume for the moment that the observed distribution may be possible under this assumption.

The Chemical Composition of Tektites.—However, if tektites arrived from the moon, we must consider possible processes by which they acquired their chemical composition. Students of this subject emphasize the great chemical similarity of tektites to terrestrial sediments.[1] These sediments get their composition by the erosion of granitic- and basaltic-type rocks in an oxidizing atmosphere containing carbon dioxide. Such an origin for the chemical properties of tektites requires most unusual assumptions in regard to the past history of the moon if the latter is to be considered as the place of origin. We can hardly assume a set of conditions on the moon in the past identical with that now existing on the earth.

The partial melting processes which produce basalt and granite of igneous origin do not produce material of the composition of tektites, and hence these processes cannot account for tektitic material on the moon. This is the conclusion of geochemists generally, and the reader should go over the analyses of igneous rocks and of tektites and decide for himself whether this is a possible source of this material. The writer has not been able to convince himself of this possibility, though he suggested some time ago that a countercurrent fractionation might have occurred during the solidification of the lunar maria which might have produced material of tektitic composition. This suggestion convinced none of his geochemical acquaintances.

Silicon, aluminum, and potassium, of the more abundant elements, are increased in concentration in the tektites relative to average meteoritic material, while magnesium and iron are decreased in abundance relative to this same material, and sodium and calcium are almost unchanged. This does not follow any pattern of separation of more volatile from less volatile elements as estimated by Urey.[2] Silicon, potassium, and sodium are markedly more volatile than aluminum, calcium, magnesium, and iron. Hence some partial evaporation or condensation process does not appear to be a feasible explanation.

The only other general physical-chemical method for the concentration of elements depends on differences in solubility. The solvent must be water, but terrestrial experience is not very informative because of the earth's oxidizing atmosphere, in which carbon dioxide is the effective agent of weathering and solution. The assumption of an oxidizing atmosphere for the moon at any time of its history is unrealistic, and in a reducing atmosphere we should expect ammonia and carbon compounds to be present. Ammoniacal water solutions should dissolve silica[3] and alumina,[4] and, moreover, potassium would remain in solution in such solutions instead of being preferentially adsorbed as compared to sodium, as occurs in terrestrial weathering, since the ammonium ion can replace potassium in clay materials. The uranium content of tektites, 1.4–1.8 p.p.m.,[5] indicates that this element has been concentrated to a high degree in these objects, as it is in the igneous rocks of the earth or in terrestrial sediments. There seems to be no very certain way by which uranium could be concentrated by a solution process. The writer has previously assumed that the moon accumulated at low temperatures along with other objects which subsequently accumulated into the earth. It was assumed that condensed water and ammonia were possible constituents of such objects. Subsequently, due to a growing acquaintance with the carbonaceous chondritic meteorites, it was further assumed that some carbon compounds might be present. If such a mixture became heated by even small amounts of radioactivity, water would flow to the surface of the moon and leach some substances from its interior; potassium, sodium, silica, and alumina would be among these. Both potassium and sodium remain in solution when an acid solution of a meteorite is precipitated with an ammoniacal solution. Heavy metals are known to form stable and soluble compounds with some carbon compounds, and uranium and thorium might be transported in some process of this kind. At higher temperatures and pressures the solubility fractionation would pass continuously into partial fusion fractionations, mentioned above. Perhaps some of the properties of a tektite might be accounted for in this way, and some transport of potassium, silica, alumina, uranium, and thorium to the surface would occur.

But it is very surprising that such a completely different process should produce material so very nearly identical with the clays of the earth. As shown in certain analyses of tektites by K. Rankama and O. Joensuu, presented by Joensuu at a conference on the abundances of the elements some two years ago, the composition of tektites even with respect to many minor constituents is that of terrestrial argillaceous sediments. At this point one becomes most doubtful of all postulated processes for producing tektitic material on the moon. One must assume a chemical process on the moon which will duplicate the results of terrestrial igneous and sedimentary processes exactly. Then this material must be removed from the moon by meteoritic collisions, melted into a glass, presumably by the energy of such collisions, and transported in a rather tight swarm of objects to the earth. One becomes skeptical in regard to the whole suggestion.

A Reconsideration of the Lunar Origin of Tektites.—An approximate formula for the minimum mean density of a swarm of objects in order that it shall be gravitationally stable in the field of the sun or earth is $\rho/\rho_0 = 8\ r_0^3/r^3$, where ρ is the density of the swarm of objects, r is their distance from the planet or sun, and r_0 and ρ_0 are the radius and density, respectively, of the sun or planet. This gives 1.1×10^{-6} gm/cm^3 for a swarm at the earth's radius in the field of the sun and 2.2×10^{-4} gm/cm^3 for one at the moon's radius in the field of the earth. The lunar swarm must have had a much smaller density than this limiting value, since the surface density of tektites is very low, and hence the swarm must have come very directly to the earth or it would have been widely scattered. This means fairly accurate aiming at the earth, and this seems improbable. A splash on the moon could hardly produce anything but a wide distribution of objects moving in all directions with some spread in velocities. Their orbits would be ellipses extending out to the moon's orbit and beyond, and the perturbation by the moon should make some of them collide with the earth from time to time. As a result, tektites would fall at all points on the earth over long periods of time. Some one splash might have been a very special one, but the tektites have been produced over a period of time since the Miocene,[1] and in some ten or more localized areas. Thus the assumption that tektites came from the moon seems to be an impossible one from purely astronomical arguments.

Conclusion.—The tektites are most reasonably regarded as terrestrial in origin, as Barnes and others have concluded.

Spencer[6] suggested that they are produced by collisions of large meteorites with the earth and that such objects melted and scattered known sedimentary materials of the proper composition. He states that objects very similar in character to tektites have been found near some meteor craters. However, no cryptovolcanic structures are known in the regions where tektites are found. Lightning striking the earth does not seem to be a likely method of production.

Is it possible that truly large cryptovolcanic structures have been overlooked? A number of small asteroids which have passed near the earth (500,000–6,500,000 miles) are known. Russell, Dugan, and Stewart[7] state that "no body of this size [a mile or two in diameter] has hit any land area on earth for *many thousands* of years" (my italics). It would seem that such collisions would be quite probable during the time since the Miocene. Such objects would have energies of about 10^{29} ergs, that is, equivalent to some hundred million atomic bombs. This energy is

sufficient to volatilize 5,000 km.^{3} of water or melt some 2,000 km.^{3} of rock. The objects would penetrate the earth for some miles. Is it possible that we do not correctly estimate the kind of effect that such objects would produce if they fell on the earth? For example, may such an object have fallen on land near the southern edge of Australia and have converted the land area into ocean area and left a cryptovolcanic structure under the present sea? Or could such an object, falling in a land area, have initiated a large plutonic event which completely obliterated any characteristic cryptovolcanic effects? It does seem that such objects might be capable of producing large numbers of small glass objects from sedimentary materials and of distributing them over wide areas.

Kuiper has recently suggested that tektites came from the moon (PROC. NATL. ACAD. SCI., **40,** 1101–1102 (1954)), which is a different view from that advanced by him previously (PROC. NATL. ACAD. SCI., **39,** 1158 (1953)), namely, that they consisted of material which had evaporated from the earth, escaped its field and moved in circum solar orbits, and that they were melted and degassed by close approaches to the sun. He advanced this latter view in a discussion with the writer in December 1953 while the writer presented the arguments of the first part of this paper. At that time I had not thought of the rather obvious astronomical difficulties presented in the latter part of the present paper. Kuiper still believes that tektites evaporated in some way from the earth in spite of the fact that their chemical composition bears no relationship to the volatile properties of their compounds.

[1] An excellent review of this subject has been given by V. E. Barnes, "University of Texas Publications," No. 3945 (1940), pp. 477–656.

[2] H. C. Urey, *Astrophys. J.*, Suppl., **1,** 147–174, 1954, and earlier papers.

[3] C. W. Correns, in T. Barth, C. W. Correns, and P. Eskola, *Entstehung der Gesteine* (Berlin: J. Springer, 1939), p. 129.

[4] K. Scharrer, *Forschungsdienst*, **1,** No. 7, 505 ff. (1936).

[5] J. A. S. Adams, private communication (1954).

[6] L. J. Spencer, *Nature*, **131,** 117–118 and 876, 1933; **132,** 571, 1933.

[7] H. N. Russell, R. S. Dugan, and J. Q. Stewart, *Astronomy, I* (New York: Ginn & Co., 1945), p. 357.

DYNAMIC PROGRAMMING AND A NEW FORMALISM IN THE THEORY OF INTEGRAL EQUATIONS

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1. *Introduction.*—In a series of papers[1, 2] and in two monographs[3, 4] we have treated various aspects of the mathematical theory of multistage decision processes, a rather imposing title which we have shortened to "dynamic programming." In several of these[5, 6] we have shown that the calculus of variations may be viewed as a multistage decision process of continuous type and that the functional equation technique which we have employed in connection with various other continuous

processes may be utilized in this discipline to yield new results and new derivations of old results in the one-dimensional case. In the process of extending these methods to include multidimensional variational problems, the theory of functional analysis enters in a very natural way, since boundary and initial values are now functions rather than numbers. It turns out that an excellent testing ground for these new tools is the theory of integral equations, which combines the new aspects of functional analysis with greater analytic simplicity than is afforded by partial differential equations. In addition, the results are of independent interest.

We shall sketch below an application of the theory of dynamic programming to the problem of obtaining a functional equation for the characteristic values of the integral equation

$$\lambda u(t) = \int_0^T K(s, t)u(s)\, ds. \tag{1.1}$$

Following this line of thought, we shall in a subsequent work develop corresponding results for some multidimensional problems in the calculus of variations.

2. *Causality and Optimality.*—As is discussed and illustrated by means of a wealth of examples in Hille's treatise,[7] a large part of analysis may be very properly, profitably, and naturally subsumed under the general theory of semigroups of operations. Here the basic functional equation for a multistage process is

$$f(P;\ s + t) = f(P(s);\ t), \tag{2.1}$$

which, as remarked by Hadamard, expresses the principle of causality.[8]

If we consider a multistage decision process, where there are many alternative routes to be pursued, the analogue of the above equation is

$$f(P;\ s + t) = \max_Q f(P(s, Q);\ t), \tag{2.2}$$

which expresses the principle of optimality.[2, 5]

As we have shown elsewhere,[4, 5] various results in the calculus of variations and in eigenvalue problems connected with differential equations may be obtained from equation (2.2) and its limiting form as $s \to 0$, the "infinitesimal generator" of equation (2.2). We now turn to the application of equation (2.2) to the theory of integral equations.

3. *Integral Equations.*—Let $K(s, t)$ be a symmetric kernel belonging to L^2 over the square $0 \leq s, t \leq T$, which is assumed to be positive definite. The characteristic values of equation (1.1) are then equivalent to the relative minima of $\int_0^T u^2\, dt$ subject to the constraint that $\int_0^T \int_0^T K(s, t)u(s)u(t)\, ds\, dt = 1$. In order to convert this minimization problem into one with the proper invariant qualities, we employ the device used earlier[5, 6] and imbed the problem within the larger problem of determining the relative minima of the functional

$$J(a,\ r(t),\ u(t)) = \int_a^T u^2(t)\, dt, \tag{3.1}$$

subject to the constraint

$$\int_a^T \int_a^T K(s, t)u(s)u(t)\, ds\, dt + 2\int_a^T r(t)u(t)\, dt = 1, \tag{3.2}$$

where $r(t) \in L^2(0, T)$. Let us for the sake of simplicity consider only the absolute minimum and define the function $f(a;\ r(t))$ as follows:

$$f(a;\ r(t)) = \operatorname{Max}_{u} J(a;\ r(t), u(t)). \tag{3.3}$$

The function f is "une fonction des lignes" in the sense of Volterra.[10]

Let us now proceed, using the principle of optimality embodied in equation (2.2) to obtain an approximate functional equation for f, under the assumptions of continuity for $K(s, t)$ and $r(t)$ as functions of s and t.

Let h be a small positive quantity. Then, for typographical convenience setting $v = u(a)$, we have

$$f(a;\ r(t)) = hv^2 + \int_{a+h}^{T} u^2\,dt + o(h), \tag{3.4a}$$

$$\int_{a+h}^{T}\int_{a+h}^{T} K(s, t)u(s)u(t)\,ds\,dt + 2\int_{a+h}^{T} [r(t) + hK(a, t)]u(t)\,dt = 1 - hr(a)v + o(h). \tag{3.4b}$$

Introducing the change of variable, $u(t) = (1 - hr(a)v/2)w(t)$, in order to normalize the relation of equation (3.4b), we see that the problem of choosing $u(t)$ in $[a + h, T]$ is a problem of precisely the same form as the original. Hence we obtain the approximate functional equation

$$f(a;\ r(t)) = \operatorname{Min}_{v} [hv^2 + (1 - hr(a)v)f(a + h);\ (r(t) + hK(a, t)(1 + hr(a)v/2))] + o(h). \tag{3.5}$$

To obtain a limiting functional equation of differential form, we must employ functional derivatives.

4. *A Gâteaux Differential Equation.*—The appropriate functional derivative to employ here is the Gâteaux derivative rather than the Volterra. We shall employ essentially the notation of Hille[9] and write $\delta_h(f)$ for the Gâteaux derivative of f with increment h:

$$\delta_h(f) = \lim_{\epsilon \to 0} \frac{f(a;\ r(t) + \epsilon h(t)) - f(a;\ r(t))}{\epsilon}. \tag{4.1}$$

Using this notation, the limiting form of equation (3.5) is

$$f_a = -\operatorname{Min}_{v} [v^2 + vr(a)\delta_{r(t)}(f) + \delta_{K(a,\,t)}(f)], \tag{4.2}$$

where f_a is the usual partial derivative. This functional differential equation may be used to provide a sequence of successive approximations to f, which can be made to converge monotonically if we choose an initial approximation in policy rather than function space.[6]

Furthermore, owing to the simple dependence of the expression in equation (4.2) upon v, the function f may be eliminated and an equation for $v = v(a;\ r(t))$, also involving Gâteaux differentials, derived. This equation will have a "characteristic theory," and, as in the case of the calculus of variations, the characteristics will be associated with the Euler equation obtained by classical variational techniques. These topics will be discussed in subsequent papers.

[1] R. Bellman, "The Theory of Dynamic Programming," these PROCEEDINGS, **38**, 716–719, 1952; "Some Functional Equations in the Theory of Dynamic Programming," *ibid.*, **39**, 1077–1082, 1953.

[2] R. Bellman, "The Theory of Dynamic Programming," *Bull. Am. Math. Soc.* (Jan., 1955).

[3] R. Bellman, *An Introduction to the Theory of Dynamic Programming* (RAND Monograph R-245, 1953).

[4] R. Bellman, *Dynamic Programming and Continuous Processes* (RAND Monograph R-271, 1954).

[5] R. Bellman, "Dynamic Programming and a New Formalism in the Calculus of Variations," these PROCEEDINGS, **40**, 231–235, 1954.

[6] R. Bellman, "Monotone Convergence in Dynamic Programming and the Calculus of Variations," *ibid.*, (these PROCEEDINGS, **40**, 1073–1075, 1954).

[7] E. Hille, *Functional Analysis and Semi-groups* ("American Mathematical Society Colloquium Publications," Vol. XXXI [1948]).

[8] Cf. *ibid.*, p. 71.

[9] *Ibid.*, p. 388.

[10] V. Volterra, *Leçons sur les fonctions des lignes* (Paris: Gauthier-Villars, 1913).

*ARITHMETIC GENERA OF NORMAL VARIETIES IN AN ALGEBRAIC FAMILY**

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It is well known that linear equivalence of divisors on a fixed ambient variety is preserved by specialization.[1] In this paper we shall show that the above assertion remains valid even when the ambient variety varies under specialization. This fact will be used as a lemma in our later paper. Here we shall derive the following theorem as an immediate consequence. If a normal variety V' is a specialization of a positive cycle V, then V is also a normal variety, and they have the same arithmetic genus. In the case of curves this assertion was proved by Chow and Nakai,[2] and in our proof we shall use some of their ideas. We note also that a slightly less general result was proved in the classical case by Spencer and Kodaira quite recently.[3]

Let V^r be a variety of dimension r in a projective space L^n. The *Hilbert characteristic function* $\chi(V, t)$ of V^r is then a polynomial in t of degree r. We shall denote its constant term by $\chi(V)$, and we call it the *arithmetic genus* of V. The usual arithmetic genus $p_a(V)$ of V is related to $\chi(V)$ by $\chi(V) = 1 + (-1)^r p_a(V)$. If V^r has no multiple subvariety of dimension $r - 1$, we can consider linear systems on V^r. The complete linear system of hypersurfaces of degree m in L^n induces a linear system L_m on V; and from the definition of Hilbert characteristic function we get

$$\chi(V, m) = \dim L_m + 1$$

from a certain m onward.

Now let V'^r be a variety in L^n of dimension r such that V' is a specialization of a positive r-cycle V^r in L^n over a field k. Since addition of cycles is compatible with specialization,[4] V is also a variety and the following lemma holds:

LEMMA 1. *A specialization of the singular locus of V over the specialization $V \to V'$ with reference to k is contained in the singular locus of V'.*

Proof: Let W^s be a component of the singular locus of V, and let W' be a specialization of W over the specialization $V \to V'$ with reference to k. We have only to show that W' is contained in the singular locus of V'. Take a field of definition K of V, V' containing k over which W^s and W' are rational. Take a generic linear

variety L^{n-s} over K with defining coefficients (u). Then the 0-cycle $W'\cdot L$ is the unique specialization of the 0-cycle $W\cdot L$ over the specialization $(V, W) \to (V', W')$ with reference to $k(u)$. Now, if W' is not contained in the singular locus of V', there exists at least one simple point P' of V' in $W'\cdot L$. Let P be a point in $W\cdot L$ which has specialization P' in the above specialization. Now take two independent generic linear varieties L^{r+1} and L^{n-r-2} with defining coefficients (v) over the field $K(u)$, and project V, P, V', and P' from L^{n-r-2} into L^{r+1} obtaining $\bar{V}$, $\bar{P}$, $\bar{V}'$, and $\bar{P}'$ respectively. We know that $(\bar{V}', \bar{P}')$ is a specialization of $(\bar{V}, \bar{P})$ over $k(v)$. Moreover, $\bar{P}$ is multiple on $\bar{V}$, while $\bar{P}'$ is simple on $\bar{V}'$. Since $\bar{V}$ and $\bar{V}'$ are hypersurfaces in L^{r+1}, we can readily get a contradiction.

In particular, if V' is nonsingular, V is also nonsingular. The following lemma is due to Chow and Nakai, but we shall give a simpler proof.

LEMMA 2. *If a nonsingular curve C' is a specialization of a curve C over a field k, they have the same genus.*

Proof: Let Δ and Δ' be the diagonals of the products $C \times C$ and $C' \times C'$. Then $(C' \times C', \Delta')$ is the unique specialization of $(C \times C, \Delta)$ over the specialization $C \to C'$ with reference to k (here we consider $C \times C$ as a subvariety of a projective space L^N with $N = (n+1)^2 - 1$ in the usual way). Now let K be a field of definition of C, C' containing k, and let L^{N-3} be a generic linear variety over K in L^N with defining coefficients (u). The projecting cones H and H' of Δ and Δ' with center L^{N-3} are hypersurfaces of the same degree, say m, in L^N such that H' is the unique specialization of H over the specialization $\Delta \to \Delta'$ with reference to $k(u)$. Now let H_0 be a generic hypersurface over $K(u)$ of degree m with defining coefficients (v). We then see that $(C' \times C', \Delta', (C' \times C')\cdot H', (C' \times C')\cdot H_0)$ is the unique specialization of $(C \times C, \Delta, (C \times C)\cdot H, (C \times C)\cdot H_0)$ over the specialization $C \to C'$ with reference to $k(u, v)$. If we put $X = (C \times C)\cdot(H_0 - H) + \Delta$ and $X' = (C' \times C')\cdot(H_0 - H') + \Delta'$, the intersection products $\Delta\cdot X$ and $\Delta'\cdot X'$ are both defined. Therefore, $\Delta'\cdot X'$ is the unique specialization of $\Delta\cdot X$ over the specialization $C \to C'$ with reference to $k(u, v)$. On the other hand, X is linearly equivalent to Δ on $C \times C$, and X' is linearly equivalent to Δ' on $C' \times C'$. Therefore,[5] we have $2\chi(C) = \deg(\Delta\cdot X) = \deg(\Delta'\cdot X') = 2\chi(C')$.

The following lemma can be proved in the usual way,[6] using a weak form of Kronecker-Castelnuovo's theorem, due to Néron-Samuel.[7]

LEMMA 3. *Let V^r be a variety in L^n without multiple subvariety of dimension $r-1$ $(r \geq 2)$. Let D be a V-divisor which is rational over a field of definition K of V. Also, let C_m be an intersection product of V with a generic hypersurface of degree m in L^n over K. Then, if $D\cdot C_m$ is linearly equivalent to zero on C_m for every m, D itself is linearly equivalent to zero on V.*

Now we can prove the following:

THEOREM 1. *We assume that a variety V'' without multiple subvariety of dimension $r-1$ is a specialization of a variety V^r over a field k. Let D be a V-divisor which is linearly equivalent to zero on V, and let D' be a specialization of D over the above specialization. Then D' is linearly equivalent to zero on V'.*

Proof: It follows from Lemma 1 that V has no multiple subvariety of dimension $r-1$. We shall show that the assertion for r follows from the corresponding assertion for $r-1$, when $r \geq 2$. In fact, take a common field of definition K of V and V' containing k over which both D and D' are rational. Let H be a generic

hypersurface of degree m in L^n over K with defining coefficients (u). Then $(V'\cdot H, D'\cdot H)$ is a specialization of $(V\cdot H, D\cdot H)$ over the field $k(u)$. We know[8] that $V'\cdot H$ is again a variety without multiple subvariety of dimension $r-2$. Since $D\cdot H$ is linearly equivalent to zero on $V\cdot H$, by induction assumption, $D'\cdot H$ is linearly equivalent to zero on $V'\cdot H$. Since this is true for every H, by Lemma 3, D' is linearly equivalent to zero on V'. We may therefore assume that V and V' are both nonsingular curves C and C'. Moreover it is quite easy to see that we have only to prove the following assertion. If Z is a member of L_m and if Z' is a specialization of Z over the specialization $C \to C'$ with reference to k, then Z' is a member of the trace L_m' on C' of the complete linear system of hypersurfaces of degree m in L^n for all m sufficiently large.

Now let Φ be the everywhere regular rational mapping of L^n into a projective space L^N which is associated with the complete linear system of hypersurfaces of degree m in L^n. Since Φ is everywhere regular, if φ and φ' are functions induced by Φ on C and C', then their graphs are related to the graph of Φ by $\Gamma_\varphi = \Gamma_\Phi\cdot(C \times L^N)$ and $\Gamma_{\varphi'} = \Gamma_\Phi\cdot(C' \times L^N)$. On the other hand, since C and C' are nonsingular, both are normal, and therefore L_m and L_m' are complete[9] from a certain m onward and have the same dimension by Lemma 2. Now let L^s be the smallest linear subvariety of L^N containing the projection of Γ_φ on L^N. We can find a hyperplane L^{s-1} of L^s such that $Z = \mathrm{pr}_C\,[\Gamma_\varphi\cdot(C \times L^{s-1})]$. Let $(\Gamma', L'^s, L'^{s-1})$ be a specialization of $(\Gamma_\varphi, L^s, L^{s-1})$ over the specialization $(C, Z) \to (C', Z')$ with reference to k. Then, first of all, we have $\Gamma' = \Gamma_{\varphi'}$ by specialization theory. Moreover, since s is equal to the dimension of L_m and hence also of L_m', we see that L'^s is the smallest linear subariety of L^N containing the projection of $\Gamma_{\varphi'}$ on L^N. Therefore, the intersection product $\Gamma_{\varphi'}\cdot(C' \times L'^{s-1})$ is defined on $C' \times L'^s$. Hence, by specialization theory,[10] we conclude: $Z' = \mathrm{pr}_{C'}\,[\Gamma_{\varphi'}\cdot(C' \times L'^{s-1})]$, completing the proof.

The following is our main theorem:

THEOREM 2. *If a normal variety V'' is a specialization of a variety V', then V is also normal and they have the same Hilbert characteristic function, hence a fortiori the same arithmetic genus.*

Proof: We can use the same notations as in the above proof, replacing C and C' by V and V'. Since L'^s contains the projection of $\Gamma_{\varphi'}$ on L^N, we get $\dim L_m \geq \dim L_m'$. On the other hand, consider the Chow varieties U and U' of the complete linear systems $|L_m|$ and L_m' for m sufficiently large. Let U^* be a specialization of U over the specialization $V \to V'$ with reference to k. Then we conclude from Theorem 1 that U^* is carried by U', whence

$$\dim U = \dim U^* \leq \dim U'.$$

However, we have

$$\dim U = \dim |L_m| \geq \dim L_m \geq \dim L_m' = \dim U';$$

hence they are all equal. Therefore, L_m is complete whenever L_m' is complete. In view of the Muhley-Zariski criterion[11] this proves the normality of V and also that both V and V' have the same Hilbert characteristic function.

COROLLARY. *If two members of a maximal algebraic family of positive r-cycles in L^n are both normal varieties, they have the same arithmetic genus.*

We note that we cannot expect a better assertion than the above one. Namely, the bunch of positive r-cycles of the same degree in L^n is connected and yet two nonsingular members of this bunch may well have different arithmetic genera.

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[1] A. Weil, *Varietés Abéliennes et courbes algébriques* ("Actualités Sci. et Ind.," No. 1064 [Paris: Hermann et Cie, 1948]), 61–62, Lemma 10.

[2] W. L. Chow, "On the Genus of Curves of an Algebraic System," *Trans. Am. Math. Soc.*, **65**, 137–140, 1949; Y. Nakai, "On the Genus of Algebraic Curves," *Kyoto Math. Mem.*, **27**, 163–165 1952.

[3] To appear shortly.

[4] Cf., e.g., T. Matsusaka, "Specialization of Cycles on a Projective Model," *Kyoto Math. Mem.*, **26**, 167–173 1951.

[5] A. Weil, *Courbes algébriques* ("Actualités Sci. et Ind.," No. 1041 [Paris: Hermann et Cie, 1948]), 13.

[6] O. Zariski, *Algebraic Surfaces* (Chelsea, New York, 1948), 88–89.

[7] A. Néron and P. Samuel, "La Variété de Picard d'une variété normale," *Ann. de l'Institut Fourier*, **4**, 1–30, 1953.

[8] This follows from the so-called first and second Bertini's theorems.

[9] O. Zariski, "Complete Linear Systems on Normal Varieties and a Generalization of a Lemma of Enriques-Severi," *Ann. Math.*, **55**, 552–592, 1952.

[10] Strictly speaking, the compatibility of the intersection product and the specialization is proved only in the case when the ambient variety is fixed. However, we can reduce our general case to this case by using projecting cones.

[11] Zariski, *Ann. Math.*, **55**, 563, 1952.

ON GENERATING FUNCTIONS FOR RESTRICTED PARTITIONS OF RATIONAL INTEGERS

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Introduction.—Suppose that $a_1, \ldots, a_n$ are nonnegative integers not all of which are zero, and consider the function

$$F(z, x, n, a_1, \ldots, a_n) = \prod_{i=1}^{n} (1 - zx^i)^{a_i}. \tag{1}$$

For $z = 1$ or $z = -1$ and $a_i = 1$, $i = 1, \ldots, n$, we have a function extensively studied.[1]

We propose to give in the first part of this paper a recursion relation (4) for the coefficients of the polynomial in x resulting from the expansion of the product displayed in (1). We follow this by specializing (1) by choosing $z = 1$ and present a theorem concerning the symmetry of the coefficients of the polynomial obtained by expanding (1). Then, taking $a_i = 1$ for $i = 1, \ldots, n$, we determine some arithmetic properties of the coefficients of this polynomial.

Pervading most of these relations is the von Sterneck number,[2]

$$\Phi(k, n) = \frac{\varphi(n)}{\varphi(n/(k,n))}\, \mu(n(k, n)),$$

where k and n are positive integers, $\varphi(n)$ is the Euler totient (or indicator), and $\mu(n)$ is the Möbius number. Also, (k, n) is the greatest common divisor of k and n.

Nicol hopes to publish later a paper concerning the combinatorial properties of these coefficients. These results will include many of the theorems of von Sterneck[3] concerning the partitions of integers with respect to a modulus.

Suppose that

$$\prod_{s=1}^{n} (1 - x^s) = \sum_{s=0}^{\hat{n}} A_s^{(n)} x^s, \tag{1a}$$

where $\hat{n} = n(n + 1)/2$.

A rather interesting problem due to Nicol is whether there is a number K such that[4]

$$|A_s^{(n)}| < K \qquad \text{for} \qquad n = 1, 2, \ldots, \qquad \text{and} \qquad 0 \leqslant s \leqslant \hat{n}.$$

In the remainder of the paper the symbol $[x]$ will denote the largest integer not exceeding x.

1. *Properties of the General Function* $F(z, x, n, a_1, \ldots, a_n)$.—Suppose that (1) is written in polynomial form as

$$F(z, x, n, a_1, \ldots, a_n) = \sum_{s=0}^{N} H_s(z) x^s, \tag{2}$$

where $N = \sum_{j=1}^{m} j a_j$. (Hereafter we shall write F, meaning $F(z, x, n, a_1, \ldots, a_n)$.)

Suppose that $0 \leqslant x < 1$ and $0 \leqslant z < 1$. Then, if m is a positive integer, we have

$$\sum_{h=0}^{m} \binom{m}{h} F^{(m-h)} D^{(h+1)}(x, z) = F^{(m+1)}, \tag{3}$$

where

$$F^{(t)} = \frac{d^t}{dx^t} (F) \qquad \text{and} \qquad D^{(t)}(x, z) = \frac{d^t}{dx^t} \log (F).$$

Since x and z are nonnegative numbers less than one, $-a_k \log (1 - zx^k) = a_k(zx^k + z^2x^{2k} + z^3x^{3k}/3 + \ldots)$. Then

$$-\sum_{k=1}^{n} \frac{d^s}{dx^s} a_k \log (1 - zx^k) = \sum_{(r)} \sum_{k=1}^{n} a_k \frac{rk \ldots (rk - s + 1)}{r} z^r x^{rk-s},$$

where the outer summation on the right is over all positive integral values of r such that $rk \geqslant s$.

Then, when $x = 0$, relation (3) would become

$$-m! H_{m-h}(z) \sum_{rk=h+1} k a_k z^r.$$

Also, when $x = 0$, $F^{(m+1)} = (m + 1)! H_{m-h}(z)$, and we have, finally:

THEOREM 1. *If* $H_s(z)$ *is defined as in* (2), *then*

$$-\sum_{h=0}^{m} H_{m-h}(z) \sum_{rk=h+1} k a_k z^r = (m + 1) H_{m+1}(z), \tag{4}$$

where the inner sum is taken over all integral solutions for which $r > 0$ *and* $n \geq k > 0$.[5]

THEOREM 2. *Suppose that* $a_1, \ldots, a_n$ *are nonnegaive integers not all of which are zero and that*

$$F(1, x, n, a_1, \ldots, a_n) = \prod_{s=1}^{n} (1 - x^s)^{a_s} = \sum_{s=0}^{N} H_s x^s,$$

where $N = \sum_{j=1}^{n} j a_j$. *Then*

$$H_p = (-1)^{S_n} H_q, \tag{5}$$

where p *and* q *are nonnegative integers such that* $p + q = N$ *and* $S_n = \sum_{j=1}^{n} a_j$.

The proof of this theorem will appear in a later paper.

2. *Properties of* (1a).—We shall now prove

THEOREM 3. *Suppose that*

$$F_n(x) = \prod_{s=1}^{n} (1 - x^s) = \sum_{s=0}^{\bar{n}} A_s^{(n)} x^s, \tag{6}$$

where $\bar{n} = n(n + 1)/2$. *Then*

$$\Phi(r, n) = -\sum_{p=1}^{l_r} p A_{pn+r}^{(n)} \tag{7}$$

where $l_r = [(n + 1)/2 - r/n]$ *and* $\Phi(r, n)$ *is the von Sterneck number.*

Proof: Consider the identity

$$F_n(x) = (1 - x^n) F_{n-1}(x).$$

Differentiating this with respect to x, we may write

$$xF_n'(x) = x(1 - x^n)F_n'(x) - nx^n F_{n-1}(x). \tag{8}$$

If x is replaced by α^k, where α is a primitive nth root of unity and k is a nonnegative integer, then (8) becomes

$$\alpha^k F_n'(\alpha^k) = -nF_{n-1}(\alpha^k). \tag{9}$$

Also, however,

$$xF_n'(x) = \sum_{s=1}^{\bar{n}} s A_s^{(n)} x^s. \tag{10}$$

Replacing x by α in (10), we have

$$\alpha F_n'(\alpha) = \sum_{r=0}^{n-1} C_r^{(n)} \alpha^r, \tag{11}$$

where

$$C_r^{(n)} = n \sum_{p=1}^{l_r} p A_{pn+r}^{(n)} + r \sum_{p=0}^{l_r} A_{pn+r}^{(n)} \tag{12}$$

and $l_r = [(n + 1)/2 - r/n]$.

We now proceed to establish the following:

LEMMA. $\sum_{p=0}^{l_r} A_{pn+r}^{(n)} = 0$, *where* $l_r = [(n+1)/2 - r/n]$.

Proof: If k is a nonnegative integer,

$$F_n(\alpha^k) = \sum_{r=0}^{n-1} B_r^{(n)} \alpha^{rk}, \qquad \text{where} \qquad B_r^{(n)} = \sum_{p=0}^{l_r} A_{pn+r}^{(n)}.$$

Then

$$B_r^{(n)} = \frac{1}{n} \sum_{k=0}^{n-1} \alpha^{-rk} F_n(\alpha^k).$$

However, $F_n(\alpha^k) = 0$ for $k = 0, 1, \ldots, n-1$. Therefore, $B_r^{(n)} = 0$ for $r = 0, 1, \ldots, n-1$, which establishes the lemma.

From (11) we may write

$$C_r^{(n)} = \frac{1}{n} \sum_{h=0}^{n-1} \alpha^{-rh} \alpha^h F_n'(\alpha^h). \tag{13}$$

Then, using (9), we may write (13) as

$$C_r^{(n)} = - \sum_{h=0}^{n-1} \alpha^{-rh} F_{n-1}(\alpha^h). \tag{14}$$

However, $F_{n-1}(\alpha^h)$ is zero if $(h, n) \neq 1$, and n otherwise.

Thus equation (14) becomes

$$C_r^{(n)} = -n \sum_{(h,n)=1} \alpha^{-rh}. \tag{15}$$

From the theorem of Hölder,[6] the last relation is

$$C_r^{(n)} = -n\Phi(r, n), \tag{16}$$

and thus Theorem 2 is established.

If n is an integer greater than one, then[7]

$$A_s^{(n)} = A_s^{(n-1)} - A_{s-n}^{(n-1)}, \tag{17}$$

where $A_{s-n}^{(n-1)}$ is zero for $s < n$.

Then, if the numbers $A_{pn+r}^{(n)}$, $p = 1, \ldots, l_r$ in (6) are replaced by $A_{pn+r}^{(n-1)} - A_{n,p-1,+r}^{(n-1)}$, it readily follows that[8]

$$\Phi(r, n) = \sum_{p=0}^{\eta_r} A_{pn+r}^{(n-1)}, \tag{18}$$

where $\eta_r = [(n-1)/2 - r/n]$.

Now consider the polynomial $F_n(x)$ defined by (6). Suppose that x is replaced by ζ, where ζ is a primitive lth root of unity and $l = n + 2$. Then

$$F_n(\zeta) = \sum_{r=0}^{l-1} D_r \zeta^r, \tag{18a}$$

where $D_r = \sum_{h=0}^{m_r} A_{h+r}^{(n)}$ and $m_r = [(n-r)/l]$. We have also, if h is a positive integer,

$$(1 - \zeta^h)(1 - \zeta^{2h}) \ldots (1 - \zeta^{h(l-1)}) = \begin{cases} l \text{ if } (h, l) = 1, \\ 0 \text{ otherwise.} \end{cases} \tag{19}$$

Then, from relations (16) and (19), we have,

$$F_n(\zeta^h) = \begin{cases} l/(1 - \zeta^{h(l-1)}) \text{ if } (h, l) = 1, \\ 0 \text{ otherwise.} \end{cases} \tag{20}$$

Consider the following known result: If $(k, l) = 1$, then $l/(\zeta^k - 1) = \zeta^k + 2\zeta^{2k} + \ldots + (l-1)\zeta^{k(l-1)}$.

Therefore, when $(k, l) = 1$,

$$\zeta^k + 2\zeta^{2k} + \ldots + (l-1)\zeta^{k(l-1)} = -(D_0 + D_1\zeta^k + \ldots + D_{l-1}\zeta^{k(l-1)}). \tag{21}$$

Then, from relations (19) and (20), upon summation of relation (21) over the set, $k = 0, 1, \ldots, -1$, and using the Hölder relation (14), we have

$$lD_k = -\sum_{s=1}^{l-1} s\Phi(s + k, l). \tag{22}$$

Replacing k by $k + 1$ in relation (22) and subtracting these equations, we may write

$$l(D_k - D_{k+1}) = l\Phi(k, l) - \sum_{s=0}^{l-1} \Phi(k + s, l),$$

since $\Phi(k + l, l) = \Phi(k, l)$.

It has previously been established[9] that

$$\sum_{s=0}^{l-1} \Phi(k + s, l) = 0.$$

Therefore, from the previous relation, we have

$$D_{k+1} = D_k - \Phi(k, l). \tag{23}$$

We have shown that, for $l > 1$,[10]

$$\sum_{s=1}^{l} s\Phi(s, l) = \frac{l\phi(l)}{2}.$$

Therefore, $D_0 = \phi(l)/2$.

Applying relation (23), we have

THEOREM 4. *Suppose that $l > 1$ and $k \geq 1$. If $\phi(l)$ is the Euler totient and $\Phi(s, l)$ is the von Sterneck number, then*

$$D_k = \frac{\phi(l)}{2} - \sum_{s=0}^{k-1} \Phi(s, l), \tag{24}$$

where D_k is defined in (18*a*).

[1] Cf. L. E. Dickson, *History of the Theory of Numbers*, Vol. 2, chap. 2. The polynomial $F(z, x, n, a_1, \ldots, a_n)$ is an extension of the polynomials considered there.

[2] *Sitzber. Akad. Wiss. Wien, Math.-naturw. Klasse*, 111 (Abt. 2*a*), 1567–1601, 1902; Bachmann, *Niedere Zahlentheorie* (Leipzig: B. G. Teubner, 1909), 2, 230–232.

[3] These PROCEEDINGS, 40, 825–835, 1954. Note literature there cited.

[4] A table of the numbers $A_s^{(n)}$, $n = 1, 2, \ldots, 34$, $0 \leq s \leq n$, was compiled by Miss C. M. Romeike. For $n = 34$, a value of $A_s^{(34)}$ was found as large as 50.

[5] By choosing z to be a complex number, it is easily shown that (4) also holds. The result stated here includes the theorem of Euler concerning the sum of divisors of a positive integer n. That is, $\sigma(n) - \sigma(n-1) - \sigma(n-2) + \sigma(n-5) + \sigma(n-7) - \sigma(n-12) - \sigma(n-15) + \ldots = 0$. Relation (4) may also be obtained using the Newton relation between sums of powers of the roots and the coefficients of this polynomial.

[6] *Prace Mat.-Fiz.*, **53**, 13–23, 1936.

[7] Cf. Bachmann, *op. cit.*, **2**, 118, 1909.

[8] These PROCEEDINGS, **39**, 963–968, 1953, Theorem 6.

[9] Bachmann, *op. cit.*, **2**, 232.

[10] These PROCEEDINGS, **40**, 825–835, 1954, Theorem 3.

ON THE TRANSCENDENTAL MODULI OF ALGEBRAIC RIEMANN SURFACES

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1. INTRODUCTION AND PRELIMINARIES

In this note I present the first by-product of a series of investigations, begun in 1951, whose purpose (as it developed) was to evaluate and apply the techniques of the calculus of variations used by Jesse Douglas[1] and others[2] on the Plateau problem to certain other problems of analysis and mathematical physics of a potential-theoretic nature. In addition to the present note, for example, these methods yield a simple theoretical treatment of the subsonic plane flow of an ideal compressible fluid about a profile.

In the course of the investigations it became necessary to devise new and more general versions of the Douglas-Dirichlet functional, using Élie Cartan's repère mobile, but this distinctive feature is not needed here.

It may be remarked that Riemann's original application of potential theory to function theory met the urgent demand for a guide to the hitherto unperspicuous calculations in the theory of the higher transcendentals. Today that motive is lacking, and a theorem in Riemannian function theory is academic per se. If it is to have any vitality at all, it must be sought in the reverse direction—that the function theory sheds light on the potential theory, and, indeed, I hope to show in the future that this is the case.

Riemann in his memoir, "Theorie der Abel'schen Funktionen,"[3] observed that, while two closed (associated with an algebraic curve) Riemann surfaces of the same genus p ($^1/_2$ the number of nonseparating closed curves or "retrosections"[4]) cannot in general be mapped conformally on one another, the totality of classes of conformal equivalent surfaces depends on $3p - 3$ ($p \geq 2$) parameters, which, since the modulus of the elliptic integral of first kind serves this function, he called "moduli." A brief résumé of his reasoning will show, however, the vagueness of this dependence. Let each such surface be represented over the z-sphere by a surface of n sheets for fixed n. Then the only variable parameters are the branch points, of which there are $2(n + p - 1)$ as is easily established by Euler's polyhedron theorem. On the

other hand, not all of these are essential, since a given surface may be mapped conformally on another n-sheeted surface by a function having polar multiplicity n. But the theorem of Riemann-Roch states that for the poles in general position there are $n - p + 1$ linearly independent functions with these poles, whence $2(n + p - 1)$ branch points *minus* n poles *minus* $n - p + 1$ functions make $3p - 3$ parameters. For $p = 1$ there is only 1 and for $p = 0$ none.

That is manifestly too vague a specification; *one would like numerical moduli—a set of numbers associated with each surface whose equality would guarantee conformal equivalence between two surfaces.*

Various attempts to construct *algebraic* invariants of the coefficients of the equations of the associated algebraic curves have been made.[5] But, quite apart from the incompleteness of these attempts, they are in the wrong direction, because the modulus question arises in all of conformal mapping theory, for example, in the mapping of plane multiply connected domains, where such invariants clearly have no meaning.

A more promising approach, therefore, is one that deals with the *transcendental invariants* which are provided by the periods of the Abelian integrals of the first kind (everywhere finite) and, to be more precise, of the *normal* integrals of the first kind, which are defined as follows. On an algebraic Riemann surface S of genus p consider a canonical system Γ of $2p$ retrosections, $\gamma_i, \delta_i, i = 1, \ldots, p$, where γ_i and δ_i are conjugate, i.e., γ_i joins the two sides of δ_i and vice versa. Then the ith normal integral of the first kind, denoted henceforth by $\int d\zeta_i$, is an everywhere finite integral on S such that $\int \gamma_i \, d\zeta_j = \delta_{ij}$. This specifies the integrals and their *normal* differentials, $d\zeta_i$, completely. The remaining periods, $\pi_{ij} = \int_{\delta_j} d\zeta_i$, are thus determined by the normalization and form a symmetric matrix (one can prove) of $p(p + 1)/2$ elements; and it seems natural to assume that they are moduli in the sense that two surfaces with the same period matrices are conformally equivalent. That is in fact the case[6], but, unfortunately, when $p \geq 4$, $p(p + 1)/2 \geq 3p - 3$, so that the π_{ij} are *superfluous* in number.

Therefore, a reasonable and long-standing conjecture is that a *suitable subset* of $3p - 3$ of the π_{ij} is a set of numerical moduli; and the confirmation of this conjecture is the main theorem—Theorem 1 below.

2. QUADRATIC DIFFERENTIALS AND NOETHER'S THEOREM

The key and unifying element is the application of the concept of quadratic differential to conformal mapping by Teichmueller.[7] An *everywhere finite analytic quadratic differential* (henceforth denoted simply by "quadratic differential"), $d\zeta^2 = a(z)\, dz^2$ (not necessarily the square of a $d\zeta$), is the product of dz^2 with an everywhere finite, complex analytic covariant tensor density $a(z)$ of weight 2, i.e., is formally invariant under change of local parameter z. Teichmueller was led to conjecture a relationship of the quadratic differentials to the modulus problem by

A. *The number of linearly independent quadratic differentials on a Riemann surface S of genus $p \geq 2$ is $3p - 3$, 1 when $p = 1$, and 0 when $p = 0$.*

Proof: A differential of the first kind has $2p - 2$ zeros (minus the Euler characteristic), and its square, $d\zeta^2$, a quadratic differential, has $4p - 4$ zeros, whence any $d\zeta_1^2$ has $4p - 4$ zeros, since the quotient is a function on S with as many zeros

as poles. The same reasoning shows that the number of independent $d\zeta^2$ is the number of independent functions with $4p - 4$ given poles, i.e., by the theorem of Riemann-Roch, $4p - 4 - p + 1 + 0 = 3p - 3$, when $p \geq 2$, since no $d\zeta$ can vanish at $4p - 4$ places. For $p = 0$ there are no finite differentials of any degree, while for $p = 1$ the square of $d\zeta_1$ is the only $d\zeta^2$.

A is merely a heuristic guide not needed here. Instead, the actual point of departure is the following special case of a theorem due to Max Noether.[8]

B. *On a Riemann surface of genus p which is nonhyperelliptic, if $p \geq 3$ one can find a basis for the quadratic differentials among those of the form $d\zeta_i\, d\zeta_j$.*

Referring to A, I note that by explicit computation one can prove the following complement:

C. *On a hyperelliptic surface the number of linearly independent quadratic differentials of form $d\zeta_i\, d\zeta_j$ is $2p - 1$.*

As an adjunct to B, I introduce the following notation: if the index i belongs to a certain subset I of $1, \ldots, p$, and j belongs to a similar J, then I will say that $(i, j) \epsilon (I, J)$. Thus B asserts the existence of I and J such that the $d\zeta_i\, d\zeta_j$, $(i, j) \epsilon (I, J)$, are a basis for the $d\zeta^2$.

3. MAIN THEOREM

THEOREM 1. *Let the normal integrals $\int d\zeta_i$ of the first kind of the Riemann surface S of genus p, which is nonhyperelliptic if $p \geq 3$, have periods π_{ij} over the δ_i of a canonical basis, and let $d\zeta_i\, d\zeta_j$, $(i, j) \epsilon (I, J)$ form a basis for the quadratic differentials on S.*[9] *If another Riemann surface S′ of genus p with normal integrals of periods π_{ij}' over δ_i' is such that*

$$\pi_{ij} = \pi'_{ij}, \qquad (i, j) \epsilon (I, J), \tag{1}$$

then S′ is conformally equivalent to S.

For the present the hyperelliptic case must be dealt with separately (see Sec. 4).

Proof of Theorem 1: According to Jordan and Möbius, one may assume that both S and S' have been represented on the canonical model of a surface of genus p, the sphere with p handles in such a way that the canonical bases have the same representatives. This gives rise to a homeomorphic mapping ϕ of S on S' such that γ_i goes into γ_i' and δ_i into δ_i'. The periods π_{ij} and π_{ij}' then *correspond under* ϕ.

One may assume ϕ differentiable as often as one pleases, and, accordingly, after introducing a conformal metric, $\lambda\, dw\, d\bar{w}$ (w a local parameter), on S', one may form the Douglas-Dirichlet integral

$$J(\phi) = {}^1/_2 \int\int_S (E + G)\, dx\, dy,$$

where, as a result of ϕ,

$$\lambda\, dw\, d\bar{w} = E\, dx^2 + 2F\, dx\, dy + G\, dy^2$$

$z = x + iy$ being a local parameter on S.

Now it follows from recent work of Morrey[10] and others on parametric double integral problems in the calculus of variations that there exists a twice-differentiable ϕ_0 which minimizes $J(\tilde{\phi})$ among all homeomorphisms $\tilde{\phi}$ which are deformable into ϕ.

Then the "method of variation of independent variables"[11] shows that $(E - G)/2 - iF$ is an analytic function of z; and, if one writes[7]

$$\lambda\, dw\, d\bar{w} = b\, dz + 2Re\,(a\, dz^2) \tag{2}$$

where $b = (E + G)/2$, $a = (E - G)/2 - iF$, then one sees immediately that $a\, dz^2$ must be invariant under change of parameter z and is, therefore, a *quadratic differential* on S. It is also clear from (2) that, *if $a\, dz^2$ is identically zero, then ϕ_0 is a conformal mapping of S on S'.*

Hence I must show that (1) implies that $a\, dz^2$ is identically zero.

For $p = 0$, condition (1) is vacuous, and there are no quadratic differentials; therefore, the conclusion is automatic.

When $p \geq 1$, I resort to the following device. According to (2), one may imbed S and S' in a one-parameter (real) family $S(t)$ of Riemann surfaces whose conformal metrics are given by

$$\lambda_t\, dw_t\, dw_t = b\, dz\, d\bar{z} + 2tRe(a\, dz^2), \tag{3}$$

where $0 \leq t \leq 1$, so that $S(0) = S$ and $S(1) = S'$. Observing that (3) may be written

$$\lambda_t\, dw_t\, d\bar{w}_t = b\, dz\, d\bar{z}\left[1 + 2tRe\left(\frac{a\, dz^2}{b|dz|^2}\right)\right], \tag{4}$$

one introduces with Teichmueller the idea of *infinitesimal quadratic differential*, written $d\zeta^2/|d\zeta_1|^2$, as the quotient of $a\, d\zeta^2$ by a differential with the indicated formal transformation properties. Hence $a\, dz^2/b|dz|^2$ is an infinitesimal quadratic differential. From a formal point of view, the product of a quadratic differential and the conjugate of an infinitesimal quadratic differential transforms like the volume element $dz \cdot d\bar{z}$ (exterior product). Therefore, following Teichmueller, one introduces their inner product,

$$(d\zeta^2,\, d\zeta_1^2/|d\zeta_2|^2) \equiv \iint_S \frac{d\zeta^2}{dz^2}\, \bar{B}\, dz \cdot d\bar{z}, \tag{5}$$

where

$$B\, dz/d\bar{z} = d\zeta_1^2/|d\zeta_2|^2.$$

Then one has the vital

LEMMA 1. *If the infinitesimal quadratic differential $d\zeta^2/|d\tilde{\zeta}|^2$ is orthogonal to every member $d\zeta^2_\nu$, $\nu = 1, \ldots, 3p - 3$, of a basis for the quadratic differentials on S, then it is identically zero.*

Proof of Lemma 1: $d\zeta^2$ may be written as $\sum_1^{\tau} a_\nu\, d\zeta_\nu^2$. Then

$$\iint_S \left|\frac{d\zeta^2}{dz^2}\right|^2 \frac{1}{b}\, dz \cdot d\bar{z} = (d\zeta^2,\, d\zeta^2/|d\tilde{\zeta}|^2) = \sum a_\nu\, (d\zeta^2_\nu,\, d\zeta^2/|d\tilde{\zeta}|^2) = 0,$$

where $b \equiv |d\tilde{\zeta}|^2/|dz|^2 > 0$, whence $d\zeta^2 \equiv 0$.

To conclude the proof of Theorem 1, it will now be sufficient to show that (1) implies that $a\, dz^2/b|dz|^2$ is orthogonal to the $d\zeta_i\, d\zeta_j$, $(i, j) \neq (I, J)$.

To that end, I note that as a consequence of (4) one may say that $S(t + \epsilon)$ is obtained from $S(t)$ by deforming its metric by the infinitesimal deformation $\epsilon a\, dz^2/b\,|dz|^2$; in other words, in the limit $S(t)$ is obtained from $S = S(0)$ by the operations of a *one-parameter group*. There remains only the task of computing the effect of this group on the π_{ij}, and that is the substance of the

VARIATIONAL THEOREM. *If the periods of the normal integrals of the first kind of $S(t)$ are denoted by $\pi_{ij}(t)$, then*

$$\pi_{ij}(t) = e^{tX_{ij}}\pi_{ij}, \tag{6}$$

where the infinitesimal transformation

$$X_{ij} \text{ is } \xi_{ij}\,\partial/\partial\pi_{ij} \text{ (not summed) and}$$

$$\xi_{ij} = \frac{\partial\pi_{ij}(0)}{\partial\epsilon} = \frac{1}{2\pi i}\iint_S \frac{d\zeta_i}{dz}\frac{d\zeta_j}{dz}\frac{\bar{a}}{b}\,dz \cdot d\bar{z}. \tag{7}$$

In this theorem the π_{ij} are treated as variables subject to the transformations of a one-parameter group expressed in the familiar symbolic form $e^{tX_{ij}}$. The form of X_{ij} is also standard.[12] I defer the proof until Section 5.

In particular, one has, using (1) and (6),

$$\pi_{ij}' = \pi_{ij}(1) = e^{X_{ij}}\pi_{ij} = \pi_{ij}; \qquad (i, j)\epsilon(I, J). \tag{8}$$

If one could conclude from (8) *that $X_{ij} = 0$, then, by* (7), *Lemma* 1, *and the hypothesis of Theorem* 1 *on (I, J), the proof of Theorem* 1 *would be complete.*

This somewhat delicate conclusion is a consequence of the fact *that a one-parameter group on one variable is equivalent to a group of translations* (see pp. 32–34 of Eisenhart, *op. cit.*, for what follows). That is to say, there exists a function ψ such that $\psi(\pi_{ij}(t)) = \psi(\pi_{ij}) + t$, so that, according to (8), when $t = 1$, one has a contradiction unless X_{ij}, i.e., ξ_{ij} is zero. This concludes the proof of Theorem 1.

4. REMARKS ON THE HYPERELLIPTIC CASE

One sees that C implies the existence on a hyperelliptic surface of genus p of $p - 2$ quadratic differentials *which are not of the* form $d\zeta_i\, d\zeta_j$ or a linear combination thereof. Hypothesis (1) of Theorem 1 would lead by the above reasoning only to the conclusion that $a\, dz^2/b\,|dz|^2$ is orthogonal to all the $d\zeta_i\, d\zeta_j$, which conclusion, after orthogonalizing a basis of the $d\zeta^2$, would lead to the conclusion that $a\, dz^2$ must be a linear combination of the aforesaid $p - 1$ $d\zeta^2$. If, then, one could prove that the deformation along such a $d\zeta^2$ as in (3) would change a hyperelliptic surface into a nonhyperelliptic one, then one could state a complement to Theorem 1, where the hypotheses would be changed to demand that both S and S' be hyperelliptic and the equality of the periods $\pi_{ij} = \pi_{ij}'$, $(i, j)\ \epsilon\ (I, J)$, where $d\zeta_i\, d\zeta_j$ form a basis for $2p - 1$ $d\zeta^2$. I do not dwell further on this point here. See footnote 16.

5. PROOF OF THE VARIATIONAL THEOREM

The only statement needing proof is (7). First, I need another lemma. As a preface, it may be remarked with Teichmueller[7] that, if one sets $t = \epsilon$ in (4) and assumes that $w_\epsilon = z + \epsilon\omega(z, \bar{z})$ for ϵ sufficiently small, then, on expanding both sides of (4) and comparing coefficients of ϵ, one finds that

$$\frac{\partial \bar{\omega}}{\partial \bar{z}} = \frac{a}{b}, \tag{9}$$

this being a local equation only, i.e., ω is not a tensor. But one can prove

LEMMA 2. *If S be canonically dissected to obtain a simply connected surface bounded by the contour γ consisting of all $2p$ retrosections run through twice in opposite directions, then* (9) *can be solved for a contravariant tensor (infinitesimal displacement) in S-γ taking on well-defined but, in general, different values at coincident points on γ.*

Proof: The proof uses a device that will be needed later, namely, the uniformization of S leading to a map of S on a non-euclidean polygon in $|z| < 1$ when $p \geq 2$, or the familiar parallelogram in the z-plane if $p = 1$. One may assume that the boundary of the polygon, $P(0)$, corresponds to γ, and I will retain the same letter for it. In and on $P(0)$, a/b is a function of z. To solve (9) I remark that $\partial\bar{\omega}/\partial\bar{z} = \partial\bar{w}/\partial z$ and then differentiate with respect to $\bar{z}$, to obtain

$$\frac{\partial^2 \bar{w}}{\partial \bar{z} \partial z} = 4\Delta\omega = \frac{\partial}{\partial z}\left(\frac{a}{b}\right),$$

which is merely Poisson's equation and is readily solved for ω. Integrating again, one obtains (9).

To prove conclusion (7) of the variational theorem, I need to introduce the elementary integral of the third kind, $\eta_{xy}(z)$,[13] which has a positive logarithmic pole of mass 1 at x and a corresponding negative one at y, and which has zero periods over the γ_i. One can then prove by contour integration and differentiation that

$$\frac{1}{2\pi i}\int_{b_i} \frac{\partial^2}{\partial y\, \partial z}\, \eta_{xy}(z)\, dz = \frac{d\zeta_i}{dy}(y). \tag{10}$$

Consequently, one derives

$$\frac{1}{2\pi i}\int_{b_j}\int_{b_i} \frac{\partial^2}{\partial y\, \partial z}\, \eta_{xy}(z)\, dz\, dy = \pi_{ij}. \tag{11}$$

Letting δ stand for a small change, one obtains

$$\delta\pi_{ij} = \frac{1}{2\pi i}\int_{b_j}\int_{b_i} \frac{\partial^2}{\partial y\, \partial z}\, \delta\, \eta_{xy}(z)\, dz\, dy. \tag{12}$$

Now I denote the polygon obtained by uniformizing $S(\epsilon)$ by $P(\epsilon)$ and its boundary (as well as the corresponding dissecting curve on $S(\epsilon)$) by γ^ϵ. Also, let $\eta_{xy}^\epsilon(z)$ be the elementary integral of the third kind on $S(\epsilon)$. Then the Cauchy residue theorem implies

$$\delta\eta_{xy}(z) - \delta\eta_{xy}(w) = (\eta_{xy}^\epsilon(z) - \eta_{xy}(z)) - (\eta_{xy}^\epsilon(w) - \eta_{xy}(w)) = \frac{1}{2\pi i}\int_{\gamma^\epsilon} [\eta_{xy}(u) - \eta_{xy}^\epsilon(u)]\, \frac{\partial \eta_{zw}}{\partial u}(u)\, du, \tag{13}$$

since $\eta_{xy}^\epsilon(u) - \eta_{xy}(u)$ is an analytic function of u without exception.

Now, if the integral in (13) is split into two parts, then the latter part is equal to the same integrand integrated over γ, thanks to Cauchy's theorem, and this integral is zero, as may be seen by remembering that one can refer back to S where γ

contains every retrosection twice in opposite directions and recalling the period normalization of $\eta_{xy}(z)$.

Furthermore, at the points of γ' which correspond to the "two sides" of one point on $S(\epsilon)$, $\eta_{xy}{}^{\epsilon}(u)$ will have the same difference (because of its definition) as $\eta_{xy}(u)$ has at corresponding points on γ, while $\partial\eta/\partial u\, du$ will have the same values.

Therefore,

$$\delta\eta_{xy}(z) - \delta\eta_{xy}(w) = \int_{\gamma'} \eta_{xy}{}^{\epsilon}(u) \frac{\partial \eta_{zw}}{\partial u}(u)\, du = \int_{\gamma'} \eta_{xy}(u - \delta u) \frac{\partial \eta_{zw}}{\partial u}(u)\, du =$$

$$- \epsilon \int_{\gamma'} \frac{\partial}{\partial u} \eta_{xy}(u)\omega(u) \frac{\partial \eta_{zw}}{\partial u}(u)\, du + 0(\epsilon^2) =$$

$$- \epsilon \int_{\gamma} \frac{\partial}{\partial u} \eta_{xy}(u) \frac{\partial}{\partial u} \eta_{zw}(u)\, \omega\, du + 0(\epsilon^2) \quad (14)$$

where $\delta u = \epsilon\omega(u)$, as follows from Lemma 2, and the estimate $0(\epsilon^2)$ is retained in the last step, since the two integrals, by Stokes' theorem, differ only by an integral over an area of order ϵ.

Substituting the extreme members of (14) in (12), one obtains

$$\delta\pi_{ij} = - \frac{\epsilon}{2\pi i} \int_{\gamma} \frac{d\zeta_i}{du} \frac{d\zeta_j}{du}\, \omega\, du + 0(\epsilon^2).$$

Using Stokes' theorem in the form

$$\int_{\gamma} \Omega = \iint_{S} d\Omega.$$

where $d\Omega$ is the exterior differential, one obtains

$$\frac{\partial \pi_{ij}(0)}{\partial \epsilon} = - \frac{1}{2\pi i} \int_{\gamma} \frac{d\zeta_i}{du} \frac{d\zeta_j}{du}\, \omega\, du = - \frac{1}{2\pi i} \iint_{S} d\left(\frac{d\zeta_i}{du} \frac{d\zeta_j}{du}\right) \omega\, du -$$

$$\frac{1}{2\pi i} \iint_{S} \frac{d\zeta_i}{du} \frac{d\zeta_j}{du}\, d\omega\, du = 0 + \frac{1}{2\pi i} \iint_{S} \frac{d\zeta_i}{du} \frac{d\zeta_j}{du} \begin{pmatrix} \bar{a} \\ b \end{pmatrix} du \cdot d\bar{u}$$

by (9). *Q.E.D.*

I should mention the fact that these calculations were patterned after analogous considerations due to Hadamard, Douglas,[14] and Schiffer.[15,16]

[1] *Am. J. Math.*, **61**, 545, 1939.

[2] Cf. R. Courant, *Conformal Mapping and Dirichlet's Principle* (New York, 1950).

[3] *Gesammelte Werke.*

[4] For general information on Riemann's theory of functions I refer here once and for all to (i) E. Picard, *Traité d'analyse*, Vol. 2 (Paris, 1926); (ii) H. Weyl, *Die Idee der Riemannschen Fläche* (Leipzig and Berlin, 1923); and (iii) R. Nevanlinna, *Uniformisierung* (Berlin, 1953).

[5] A. von Brill and M. Noether, "Über die algebraischen Functionen und ihre Anwendung in der Geometrie," *Math. Ann.*, **7**, 269, 1874. See also K. Hensel and G. Landsberg, *Theorie der algebraischen Funktionen* (Leipzig, 1902).

[6] Cf. F. Severi, *Vorlesungen über algebraische Geometrie* (Leipzig, 1921), Anhang I.

[7] *Abhandl. Preuss. Akad. Wiss.*, Jahrgang 1939, No. 22, 1940.

[8] Cf. Hensel and Landsberg, *op. cit.*, p. 502.

[9] I use B implicitly here.

[10] *Ann. Math.*, **49**, 807, 1948. λ may be chosen as differentiable as one pleases. In order to insure equi-continuity of a minimizing sequence of the ϕ's introduced below one must fix the images of three points when $p = 0$ and one point when $p = 1$. This is essentially due to the existence of 3 and 1 parameter groups of conformal self-transformations in these cases.

[11] T. Rado, *On the Problem of Plateau* (Berlin, 1933), p. 88.

[12] Cf. L. P. Eisenhart, *Continuous Groups of Transformations* (Princeton, 1933).

[13] For an elegant summary of these matters see M. Schiffer, *Am. J. Math.*, **68**, 444, 1946.

[14] *Ann. Math.*, **40**, 205, 1939.

[15] Courant, *op. cit.*, Appendix.

[16] The hyper-elliptic case will be dealt with in a separate forthcoming publication.

ON MONOTONIC FUNCTIONS WHOSE SPECTRUM IS A CANTOR SET WITH CONSTANT RATIO OF DISSECTION

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Let P be the perfect set of the Cantor type and of constant ratio of dissection constructed on the interval $0 \leq x \leq 2\pi$ with the ratio of dissection $(\xi, 1 - 2\xi, \xi)$, where $0 < \xi < 1/2$. We write $\xi = 1/\theta$ and denote the set P by $P(\theta)$. Then, if x is a point of P,

$$x = 2\pi[\epsilon_1(1 - \xi) + \epsilon_2\xi(1 - \xi) + \ldots + \epsilon_k\xi^{k-1}(1 - \xi) + \ldots]$$
$$= 2\pi(\theta - 1)\left[\frac{\epsilon_1}{\theta} + \frac{\epsilon_2}{\theta^2} + \ldots\right] = 2\pi\sum_1^\infty \epsilon_k r_k,$$

where $\epsilon_k = 0$ or 1 and $r_k = (\theta - 1)/\theta^k$.

By the "Lebesgue function" having P as spectrum we denote, as usual, the function defined by

$$y = f(x) = \frac{\epsilon_1}{2} + \frac{\epsilon_2}{2^2} + \ldots,$$

whenever $x \in P$, continuous, and constant in every interval contiguous to P.

Let S be the set of the algebraic integers ω larger than 1 and such that all the conjugates of ω, except ω itself, have their moduli strictly inferior to 1.

It is known[1] that the Fourier-Stieltjes coefficient

$$\frac{1}{2\pi}\int_0^{2\pi} e^{nix}\,df(x)$$

of the Lebesgue function having spectrum $P(\theta)$ tends to zero if and only if θ does not belong to S.

The question of knowing whether, if θ belongs to S, the Fourier-Stieltjes coefficient $(2\pi)^{-1}\int_0^{2\pi} e^{nix}\,dF(x)$ does not tend to zero for every function of bounded variation $F(x)$ having spectrum $P(\theta)$ (or part of P) is open[2] (except when θ is rational or quadratic). We propose to bring here a contribution to the solution of this problem.

Let us observe that it will be sufficient to confine ourselves to monotonic (nondecreasing) functions F, since, by the theorem of Rajchman-Gruzewska,[3]

$$\int_0^{2\pi} e^{nix}\, dF \to 0$$

implies

$$\int_0^{2\pi} e^{nix}\, dQ \to 0,$$

Q being the positive variation of F.

Hence, from now on, F will denote a nondecreasing, continuous function, constant in every interval contiguous to P, and we shall suppose, as we may, $F(0) = 0$, $F(2\pi) = 1$. Evidently we can write $F(x) = G[f(x)]$, where $G(y)$ is a continuous, nondecreasing function, such that $G(0) = 0$, $G(1) = 1$, and we shall adopt this notation throughout the paper.

THEOREM I. *If the Fourier-Stieltjes coefficients of dG, $\int_0^1 e^{2\pi niy}\, dG(y)$ tend to zero, then $\int_0^{2\pi} e^{nix}\, dF \neq O(1)$ whenever $\theta \in S$.*

Proof: Write

$$c_n = \int_0^{2\pi} e^{nix}\, dF(x) = \int_0^{2\pi} e^{nix}\, dG[f(x)].$$

If $x \in P$, the correspondence between x and $y = f(x)$ is one-to-one (except for the end-points of a contiguous interval); hence we can take $y = f(x)$ as new variable. If $\{\varphi_k\}$ $(k = 1, 2, \ldots)$ denotes the system of Rademacher functions, one has

$$\epsilon_k = \frac{1 - \varphi_k(y)}{2} \qquad \left(y \neq \frac{p}{2^q}\right),$$

whence

$$x = 2\pi \sum_1^\infty \frac{1 - \varphi_k(y)}{2} r_k = \pi \sum_1^\infty r_k - \pi \sum_1^\infty \varphi_k(y) r_k$$

$$= \pi - \pi \sum_1^\infty \varphi_k(y) r_k.$$

Thus

$$c_n = e^{ni\pi} \int_0^1 e^{-ni\pi \sum_1^\infty \varphi_k r_k}\, dG(y).$$

(In particular, for the Lebesgue function, $F \equiv f$, $G(y) \equiv y$, and, due to the independence of the Rademacher functions, we find immediately the usual formula $e^{ni\pi} \prod_{k=1}^{\infty} \cos \pi n r_k$.[1])

In order to prove that c_n does not tend to zero, it is sufficient to prove, replacing n by a continuous parameter u, that the integral

$$\gamma(u) = \int_0^1 e^{ui\pi \sum_1^\infty \varphi_k r_k}\, dG(y)$$

does not tend to zero as $u \to \infty$.[1]

Choose $u = 2\theta^m/(\theta - 1)$, where m is a positive integer. Then the exponent of e becomes

$$2\pi i\left[\varphi_1\theta^{m-1}+\varphi_2\theta^{m-2}+\ldots+\varphi_m+\frac{\varphi_{m+1}}{\theta}+\ldots\right].$$

Now, by a property of the numbers θ of the class S one has, δ being a constant depending on θ only,

$$\theta^h \equiv \eta_h \pmod 1, \qquad \text{with} \qquad |\eta_h| < \delta^h \ (0<\delta<1),$$

so that, if $m > h$,

$$|\gamma(u)| > \left|\int_0^1 e^{2\pi i \sum_{k=-h}^{k=h} \varphi_{m-k}\theta^k}\, dG(y)\right| - \rho_h,$$

ρ_h tending to 0 as $h \to \infty$, uniformly in $m > h$.

The integral can be written

$$\int_0^1 \prod_{k=-h}^{k=h} (\cos 2\pi\theta^k + i\varphi_{m-k}\sin 2\pi\theta^k)\, dG(y).$$

If we expand the product and integrate, we see that, except for the constant term which gives $\prod_{-h}^{h} \cos 2\pi\theta^k$, all integrals (whose number depends on h only) are of the form $\int_0^1 \psi(2^{m-h-1}y)\, dG(y)$, ψ being a product of Rademacher functions depending on h only. Now it is well known that, if the Fourier-Stieltjes coefficients of dG tend to zero, then $\int_0^1 \lambda(ny)\, dG \to \int_0^1 \lambda(y)\, dy$ as $n \to \infty$, λ being any characteristic function of interval.[4] Hence, as $m \to \infty$, $\int_0^1 \psi(2^{m-h-1}y)\, dG \to 0$. Thus, as $u = 2\theta^m/(\theta-1) \to \infty$,

$$\liminf |\gamma(u)| \geq \left|\prod_{-h}^{h} \cos 2\pi\theta^k\right| - \rho_h.$$

Hence

$$\liminf |\gamma(u)| \geq \left|\prod_{-\infty}^{\infty} \cos 2\pi\theta^k\right| \qquad \left(u = \frac{2\theta^m}{\theta-1}\right),$$

by letting h tend to infinity. Since the infinite product is a positive number when $\theta \in S^1$ (the exception $\theta = 4$ has no interest for us), we have proved that $\gamma(u)$ does not tend to zero as $u \to \infty$; hence our theorem.

In particular, the theorem proves that, if the "outer function" G is absolutely continuous, then the Fourier-Stieltjes coefficients of $dG(f)$ do not tend to zero.

Any function of bounded variation ϕ such that the Fourier-Stieltjes coefficients of $\psi(\phi)$ do not tend to zero whenever ψ is absolutely continuous has been called by Schreider "a function of the class J."[5] Thus we can say, as a consequence of our theorem, that the Lebesgue function constructed on the set $P(\theta)$, where $\theta \in S$, belongs to the class J.

THEOREM II. *If the absolutely continuous component of G does not vanish identically, then $\int_0^{2\pi} e^{nix}\, dF \neq o(1)$ whenever $\theta \in S$.*

We shall base the proof of this theorem on two lemmas, the first being a generalization of the Rajchman-Gruzewska theorem.[3]

LEMMA I.[6] *Let $\phi(x)$ $(0 \leq x \leq 2\pi)$ and $K(y)$ $(0 \leq y \leq 1)$ be both bounded, non-decreasing. We suppose that $\phi(0) = 0$, $\phi(2\pi) = 1$. If $\int_0^{2\pi} e^{nix}\, d\phi(x) \to 0$, and if $K(y)$ is absolutely continuous, then $\int_0^{2\pi} e^{nix}\, dK[\phi(x)] \to 0$.*

The theorem of Rajchman-Gruzewska states that if $\int_0^{2\pi} e^{nix}\, d\phi(x) \to 0$, then $\int_0^{2\pi} e^{nix} B(x)\, d\phi(x) \to 0$, here $B(x)$ is any bounded function. We observe first that in this result we can replace the bounded function $B(x)$ by any function $W(x)$ such that $\int_0^{2\pi} W(x)\, d\phi(x)$ exists in the Lebesgue-Stieltjes sense ($W \in L_\phi$). In fact, this implies (since step functions are dense in L_ϕ)[7] that, ϵ being given, arbitrarily small, there exists a trigonometric polynomial $P(x)$ such that

$$\int_0^{2\pi} |W(x) - P(x)|\, d\phi(x) < \epsilon.$$

But $\int_0^{2\pi} e^{nix} P(x)\, d\phi(x) \to 0$ as $n \to \infty$, hence also $\int_0^{2\pi} e^{nix} W(x)\, d\phi(x) \to 0$.

Now, if $K(y)$ is absolutely continuous, and $K'(y)$ denotes its derivative, one has

$$\int_0^{2\pi} e^{nix}\, dK[\phi(x)] = \int_0^{2\pi} e^{nix} K'[\phi(x)]\, d\phi(x),$$

and the lemma follows from the preceding remark, since $\int_0^{2\pi} K'[\phi(x)]\, d\phi(x)$ exists in the Lebesgue-Stieltjes sense.

LEMMA II. *Let $G(y)$ be continuous, nondecreasing $(0 \leq y \leq 1)$, and let, to fix the ideas, $G(0) = 0$, $G(1) = 1$. Write $G = G_1 + G_2$, where G_1 is absolutely continuous, and G_2 is purely singular. If G_1 does not vanish identically, there exists a function $H(z)$ absolutely continuous for $0 \leq z \leq 1$, such that $H[G(y)]$ is absolutely continuous.*

Since G_1 is not identically zero, there exists a set of positive measure where $dG_1/dy > 0$, hence also a closed set E, with positive measure, such that, if $y \in E$,

(1) dG_1/dy exists and $0 < \delta \leq (dG_1/dy) \leq \Delta < \infty$;

(2) dG_2/dy exists and is zero.

The change of variable $z = G(y)$ transforms $E(y)$ in a set $E_1(z)$ with positive measure, since

$$|E_1| = \int_E dG \geq \int_E dG_1 = \int_E \frac{dG_1}{dy}\, dy \geq |E|\, \delta.$$

Let $\chi(y)$ be the characteristic function of E, $\chi_1(z)$ the characteristic function of E_1. And let G^{-1} be the inverse function of G. The function

$$H(z) = \int_0^z \chi_1(u)\, dG^{-1}(u) \qquad (0 \leq z \leq 1)$$

is well determined, as the points of discontinuity of G^{-1} are not in E_1. Since, if $u \in E_1$, $1/\Delta \leq (dG^{-1}/du) \leq (1/\delta)$, $H(z)$ is absolutely continuous and not identically zero. Now

$$H[(G(y)] = \int_0^{G(y)} \chi_1(u)\, dG^{-1}(u) = \int_0^y \chi(v)\, dv,$$

by writing $G^{-1}(u) = v$. Hence $H[G(y)]$ is an absolutely continuous function of y (and even belongs to Lip 1).

Proof of Theorem II: Let $F = G(f)$, the notations being the same as in Theorem I. Here f is the Lebesgue function constructed on $P(\theta)$, and G has a nonvanishing absolutely continuous component.

Choose $H(z)$ absolutely continuous, according to Lemma II, such that $H[G(y)]$ is absolutely continuous.

Suppose that $\int_0^{2\pi} e^{nix}\, dF \to 0$. Then, by Lemma I, $\int_0^{2\pi} e^{nix}\, dH(F) \to 0$. But

$$H(F) = H[G(f)] = T(f),$$

where $T(y)$ is absolutely continuous. Hence $\int_0^{2\pi} e^{niz}\, dT(f)$ would tend to zero, which is impossible by Theorem I. This contradiction proves the theorem.

Lemma II proves that, according to the definition of Schreider, a function $G(y)$ defined as in Lemma II, with a nonvanishing absolutely continuous component, cannot belong to the class J.

We can generalize Theorem II as follows:

THEOREM III. *The notations being the same as in Theorems I and II, if G does not belong to the class J, then $\int_0^{2\pi} e^{niz}\, dF \neq o(1)$ whenever $\theta \in S$.*

The proof is very similar to that of Theorem II. Since G does not belong to J, choose $H(z)$, absolutely continuous, such that the Fourier-Stieltjes coefficients of $dH[G(y)]$ tend to zero. Let $H[G(y)] = T(y)$.

Suppose that $\int_0^{2\pi} e^{niz}\, dF \to 0$. Then, by Lemma I, $\int_0^{2\pi} e^{niz}\, dH(F) \to 0$. But

$$H(F) = H[G(f)] = T(f).$$

By Theorem I, $\int_0^{2\pi} e^{niz}\, dT(f) \neq o(1)$. Hence a contradiction, which proves the theorem.

Summing up the results, we see that, if f is the Lebesgue function constructed on $P(\theta)$, with $\theta \in S$, every function $F(x) = G[f(x)]$ has Fourier-Stieltjes coefficients not tending to zero, if G does not belong to the class J.

The case $G \in J$ remains open. If the same result held in this case also, it would be proved that every function of bounded variation having spectrum $P(\theta)$ (or part of it) with $\theta \in S$ has Fourier-Stieltjes coefficients not tending to zero. In other words, $P(\theta)$, when $\theta \in S$, would be a "set of uniqueness in the broad sense" (terminology of N. Bary).

Remark: The method used to prove Theorem I can be applied to the construction of an explicit example of the two continuous non-decreasing functions f and F having both the same perfect as spectrum (hence identical intervals of constancy) and such that the Fourier-Stieltjes coefficients of f do tend to zero while the Fourier-Stieltjes coefficients of F do not tend to zero.

Let $\{a_k\}_1^\infty$ be an infinite sequence of real numbers each of which is essentially less than 1 in absolute value, and let $\{\phi_k(y)\}_1^\infty$ be the sequence of Rademacher functions. Then, assuming that the series $\sum u_n$, where

$$u_n = \prod_1^n \left(\frac{1 + |a_k|}{2} \right),$$

is convergent, the sequence of functions

$$G_n(y) = \int_0^y (1 + a_1\varphi_1) \ldots (1 + a_n\varphi_n)\, dy$$

tends uniformly to a continuous function $G(y)$, strictly increasing from 0 to 1, which is purely singular when $\sum a_k^2 = \infty$. Moreover, the expansion of the Fourier-Stieltjes series of dG in the Walsh-Paley system is given formally by the infinite product $\prod_1^\infty (1 + a_k\phi_k)$.[8]

Take now the set $P(\theta)$ of the beginning of this paper, with $\theta = \sqrt{5}$. Since θ is not in S, the Fourier-Stieltjes coefficient

$$(2\pi)^{-1} \int_0^{2\pi} e^{niz}\, df(x)$$

of the Lebesgue function having spectrum $P(\theta)$ tends to zero. Take now the function $F(x) = G(f)$, where G is the continuous, purely singular, strictly increasing function whose Fourier-Stieltjes series in the Walsh-Paley system is $\prod_1^\infty(1 + a_k\phi_k)$, when $a_{2p} = 0$, and a_{2p+1} is positive, less than 1, and tends to 1 as $p \to \infty$, in such a way that $\Sigma(1 - a_{2p+1}) < \infty$. Plainly the condition about the convergence of $\sum u_n$ is satisfied.

Now we have, as in the proof of Theorem 1,

$$\gamma\left(\frac{2\theta^m}{\theta - 1}\right) = \int_0^1 e^{2\pi i[\varphi_1\theta^{m-1} + \varphi_2\theta^{m-2} + \ldots + \varphi_m + (\varphi_{m+1}/\theta) + \ldots]}\, dG(y),$$

and, obviously,

$$\left|\gamma\left(\frac{\phi^m}{\theta - 1}\right)\right| > \left|\int_0^1 e^{2\pi i \sum_{q=1}^{q=m+h} \varphi_q\theta^{m-q}}\, dG(y)\right| - O\left(\frac{1}{\sqrt{5}}\right)^h,$$

where the integral can be written

$$\int_0^1 \prod_{q=1}^{q=m+h} (\cos 2\pi\theta^{m-q} + i\varphi_q \sin 2\pi\theta^{m-q}) \prod_0^\infty (1 + a_{2p+1}\varphi_{2p+1})\, dy.$$

Choosing m even, h odd, the modulus of the integral is larger than

$$\left|\prod_1^\infty \cos\frac{2\pi}{5^k}\right| \cdot \left|(\cos 2\pi\theta^{m-1} + ia_1 \sin 2\pi\theta^{m-1})(\cos 2\pi\theta^{m-3} + ia_3 \sin 2\pi\theta^{m-3}) \ldots (\cos 2\pi\theta^{-h} + ia_{m+h} \sin 2\pi\theta^{-h})\right|.$$

Now

$$|\cos\varphi + ia_k \sin\varphi| = \sqrt{\cos^2\varphi + a_k^2 \sin^2\varphi} = \sqrt{1 - (1 - a_k^2)\sin^2\varphi} > \sqrt{1 - 2(1 - a_k)},$$

and thus, if h is fixed and $m \to \infty$,

$$\liminf \left|\gamma\left(\frac{2\theta^m}{\theta - 1}\right)\right| > A - O\left(\frac{1}{\sqrt{5}}\right)^h$$

A being a positive constant. Thus the Fourier-Stieltjes coefficients of dF do not tend to zero. Observe that $f = G^{-1}(F)$, where G^{-1} is also purely singular.

[1] R. Salem, "Sets of Uniqueness and Sets of Multiplicity," *Trans. Am. Math. Soc.*, **54**, 218–228, 1943.

[2] R. Salem, "Rectifications to the Papers 'Sets of Uniqueness and Sets of Multiplicity. I and II,'" *Trans. Am. Math. Soc.*, **63**, 595–598, 1948.

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[4] This remark is due to Rajchman. It is easily proved by writing $\lambda_1(y) < \lambda(y) < \lambda_2(y)$, where λ_1 and λ_2 are two trapezoidal functions such that $\int_0^1(\lambda_2 - \lambda_1)dG$ is arbitrarily small, and by using the fact that λ_1 and λ_2 have absolutely convergent Fourier series.

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[7] A. Zygmund, *Trigonometrical Series* (Warsaw-Lwów, 1935).

[5] See R. Salem, "On Some Singular Monotonic Functions Which Are Strictly Increasing," *Trans. Am. Math. Soc.*, **53**, 427–439, 1943, particularly pp. 431–433, where the notations are slightly different. The fact that the infinite product is the Fourier-Stieltjes series of dG in the Walsh-Paley system is an immediate consequence of the uniform convergence of G_n and of the fact that each function of the system is a step function.

*RADIOAUTOGRAPHIC STUDIES OF KERATIN FORMATION**

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Some general aspects of the problem of keratin formation in vertebrate tissues have been discussed briefly in a recent note.[1] One of the questions raised therein was concerned with whether keratin occurred as the result of a specific synthesis of fibrous protein or of the degradation of normal protoplasmic proteins. Data relevant to this question have been selected from some detailed radioautographic studies of the distribution of S^{35}-labeled *l*-cystine in the mouse, with special attention to the production of "hard" and "soft" keratins.[2] Additional experiments with P^{32}-labeled phosphate and C^{14}-labeled carbonate and protein-hydrolyzate were also conducted. Unless otherwise indicated, young mice of the C_{57} strain were employed. Tissues were fixed in Bouin's fluid, and paraffin sections were cut at 10 μ. The radioactive materials thus demonstrated are considered to be protein-bound. The radioautographic methods were essentially those of Bélanger and Leblond[3] (emulsion-coating technique) and of Doniac and Pelc[4] (stripping-film technique).

It was reported[1] that during the active growth phase (anagen) in the hair cycle of the mouse there occurred a striking concentration of cystine (and/or its derivatives)[5] in the hair follicles evident in radioautographs of skin removed 8 hours after the administration of S^{35}-labeled cystine. A similar high uptake was seen in the conical papillae of the mouse tongue[1] and has also been found in the claw bed. The intensive concentration of radiocystine occurs in the so-called "keratogenous zone," characteristic of "hard" keratinization.[2] This concentration could conceivably result from either specific uptake of cystine from the body pool by the keratogenous zone itself or uptake in the hair bulb associated with protein synthesis accompanying cell proliferation and subsequent retention of the cystine as the living cells keratinize and die. Radioautographs of skin made 8 hours after radiocystine injection (approximately 3 μc/gm body weight) show a definite though minimal uptake in the hair bulb (Fig. 1). However, any increase in cellularity in the keratogenous zone resulting from a decrease in volume of the cells which have proliferated from the hair bulb is insufficient to account for the manifold difference in radioactivity.

Examination of radioautographs made 1, 2, 4, and 6 hours after radiocystine administration reveals that there is an appreciable uptake of isotope by the keratogenous zone even 1 hour after administration, when the hair-bulb region is only

slightly active radioautographically (Fig. 4). The intensity of the radioautograph increases with the time after administration up to 8 hours, and the radioactive region of the hair follicle also becomes somewhat greater in extent (explicable on the basis of the growth of the hair shaft and the incorporation of the cystine therein). Essentially, however, the radioautograph appears much the same 1 hour after administration as it does 8 hours after. In mice (C_{57} and A strains) wherefrom skin and tongue sections are made one or more days after radiocystine injection, the radioactive zone can be followed out distally as the hair shaft or conical papilla grows (Figs. 2 and 3).

Sections of skin biopsied or necropsied 1–8 hours after cystine injection were treated before being covered with stripping film with various agents to determine the mode of binding of the cystine taken up by the keratogenous zone. Extraction of tissue sections with 1 and 2 per cent thioglycollate solution (at pH 10 and 11) and 0.5 *M* mercaptoethanol (at pH 4 and 7), all at 50° C. for 2 hours, revealed no apparent decrease in radioactivity when compared with distilled water–treated control sections (Figs. 5 and 6). Hence the cystine is evidently not bound to side groups by disulfide linkages but is presumably incorporated (as cysteine?) into the main chains of the keratin molecule by peptide linkages.

Eight hours after injection of about 40 μc of C^{14}-labeled algal protein-hydrolyzate into a young (15-gm.) mouse in anagen, skin sections produced lightly positive radioautographs when exposed to film for 8 or more weeks. The C^{14}-labeled amino acids were not specifically concentrated in the keratogenous zone. Activity was seen in the hair bulb and extended up the hair follicle into the keratogenous zone region with qualitatively little difference in concentration (Fig. 9). Similar results were obtained in another mouse 6 hours after injection with 300 μc of radiocarbonate (after exposure of sections to film for 9 weeks).

Eight hours after administration of massive doses of P^{32} (200–500 μc per animal), the amount of radioactivity is high in the hair bulb and decreases toward the keratogenous zone (Fig. 7). The radioautographic distribution is in direct contrast to the picture of cystine localization in the hair follicle. The radioactivity with P^{32} is concentrated in the region of active cell division and, hence, nucleic acid synthesis. Autographs made 18 and 24 hours after P^{32} injection show a generalized uptake by the lower part of the follicle, including the keratogenous zone, which is still less active than the hair bulb.

We have previously reported[1, 6] that there is little evidence of difference in the localization of cystine in the androgen-stimulated vagina and uterus as compared with the estrogen-stimulated organs, beyond that related to the degree of hyperplastic response induced in the epithelium by the steroids used. As a part of a study of the distribution of radiocystine in the female genital tract under various hormonal stimuli, 6 virgin female Heston A mice, 5 months of age and castrated for 90 days, were injected intramuscularly with estradiol in sesame oil, 0.01 mg. daily for 3 days. Eight hours before sacrifice, 3 μc of radiocystine per gram of body weight were injected subcutaneously. Segments of vagina and uterus were removed from each animal, fixed together in Bouin's fluid, and treated together thenceforth. Paraffin sections of a single block containing both tissues were made at 6 μ and were covered with stripping film. After exposure for 3 weeks, the slides were developed and the contact radioautographs studied.

Grain-counting on the superimposed film was performed at 1,500× with an ocular grid, the counts being made from twenty areas of each tissue region and recorded as mean count per grid square. Among other regions, counts were made over the stratum basale and the mid-stratum spinosum of the keratinized hyperplastic vaginal epithelium and over the lining and the glandular epithelium of the hypertrophic uterus. Table 1 gives the mean ratios of some of the regions compared. Statistical analysis of paired data shows that the radiocystine concentration in the uterine lining or glandular epithelium is not significantly different from that in the vaginal stratum basale. However, the vaginal stratum spinosum contains significantly more cystine than the other regions, although the difference between this region and the uterine lining epithelium is not great. An appreciable concentration of radiocystine is evident immediately below the keratin layer in the vagina (Fig. 8).

TABLE 1

MEAN RATIO OF GRAIN COUNTS OF RADIOCYSTINE UPTAKE BY VARIOUS REGIONS OF THE ESTROGEN-STIMULATED FEMALE MOUSE REPRODUCTIVE TRACT

Areas Compared	Mean Ratio of Counts $\pm$S.E.$_m$, n = 6
Vaginal stratum basale / Uterine lining epithelium	0.92 $\pm$ 0.03
Vaginal stratum spinosum / Vaginal stratum basale	1.28 $\pm$ 0.08
Vaginal stratum spinosum / Uterine lining epithelium	1.14 $\pm$ 0.04
Uterine lining epithelium / Uterine glandular epithelium	1.19 $\pm$ 0.06

From these data it appears that there is no specific uptake of cystine by the basal layer of the keratinizing vaginal epithelium, the radioactivity of which is indistinguishable from the nonkeratinizing uterine epithelium. However, the higher concentration in the lower and middle stratum spinosum cannot be explained on the basis of increased cellularity, since the cells of this region are at least as large as those of the stratum basale. Radioactivity is least in the stratum basale and greatest immediately below the keratin layer. Such a gradient is also seen in the "soft" keratinization occurring on the surface of the tongue (between the papillae)[1], in the foot pad, and in the lining of the mouse esophagus and forestomach. Furthermore, examination of autographs of the vagina made 4 hours after radiocystine injection into estrogen-treated female mice reveals a distribution of the cystine similar to that seen 8 hours after injection. Twenty-four hours after radiocystine administration, the basal region of the keratin layer becomes radioactive, and vaginal smears taken at 48 hours show considerable activity.

Additional information on the radioautographic localization of protein-synthetic regions with radiocystine will be published elsewhere. The technique has permitted the acquisition of some suggestive data on the mechanism of keratin formation not heretofore available. Although the differences between "hard" and "soft" keratinization are considerable[2] (recent work indicates that intermediate situations also exist[7]), the highly radioactive zone beneath the stratum corneum

in the vagina and elsewhere may be analogous to the keratogenous zone in "hard" keratinization. The higher radioactivity in this region could be a reflection of the flattening of the cells and decrease in their volume, prior to keratinization. However, this activity is present within a short time after radiocystine administration, suggesting that it need not be ascribed to the S^{35} content of cells which have proliferated from the basal layer. Less than 8 hours after isotope administration, the entire stratified epithelium is radioactive, not just the more basal layers. As has been stated previously[1] in regard to the hair follicle, the region of cystine concentration corresponds to the region of high concentration of protein-bound sulfhydryl groups (as detected cytochemically). This is also the case in the lingual papilla, as well as in the analogous area in "soft" keratinization. It would seem, then, that much of the S^{35} is present as protein-bound cysteine in the keratogenous zones.

The specific concentration ability of the keratogenous zone may be restricted to cysteine-cystine and/or their derivatives (and possibly other sulfur-containing amino acids[8]). The radiocarbon studies indicate an activity at 8 hours in this zone, which is no higher than that of the hair bulb and, hence, which could result in part from amino acids absorbed by the hair bulb and there incorporated into protein and in part by concentration of C^{14}-labeled cystine from the body pool. The radiophosphate studies show slight grain deposition over the mitotically inactive keratogenous zone 8 hours after injection of P^{32}, which may be accounted for largely by the residual DNA and RNA carried up by the cells deriving from the hair bulb.

"Enrichment in sulfur"[7] certainly occurs in the course of "hard" keratinization. That it is the one generally valid criterion for this phenomenon, as suggested by Van Scott and Flesch,[7] may be open to some question, since the "soft" keratinization in the vagina and elsewhere may also involve cystine concentration immediately beneath the keratin layer in a region analogous to the keratogenous zone. That the

hb = Hair bulb; *kz* = Keratogenous zone; *hs* = Hair shaft; *m* = Melanin.

FIG. 1.—Skin from female C_{57} mouse to show intense uptake in keratogenous zone and minimal uptake in hair bulb 8 hours after injection of S^{35}-cystine. Distinguish dense melanin in core of hair bulb and shaft from granular-appearing radioactive areas in this figure and in Figures 4–7 and 9. Overexposed emulsion coating, unstained. ×65.

FIG. 2.—Tongue from female Heston A mouse to show intensely active conical papillae 3 days after injection of S_{35}-cystine. Stripping film, unstained. ×130.

FIG. 3.—Skin from old estrogen-treated female Cal A mouse to show radioactive zone emerging from hair follicle 5 days after injection of S^{35}-cystine. Emulsion coating, unstained, uncleared. ×65.

FIG. 4.—Skin from female C_{57} mouse to show active keratogenous zone 1 hour after injection of S^{35}-cystine. Stripping film, unstained. ×65.

FIG. 5.—Hair follicles from female C_{57} mouse 8 hours after injection of S^{35}-cystine. Control for treatment with disulfide-reducing agents (Fig. 6). Note active keratogenous zones (*at right*). Stripping film, unstained, uncleared. ×65.

FIG. 6.—Hair follicles from skin section adjacent to that shown in Fig. 5. Treated with sodium thioglycollate (see text). Note retention of activity in keratogenous zones. Stripping film, unstained, uncleared. ×65.

FIG. 7.—Hair follicles from skin of male C_{57} mouse 8 hours after injection of P^{32}-phosphate. Note concentration in hair bulb decreasing toward keratogenous zone. Stripping film, unstained ×65.

FIG. 8.—Section of keratinized vagina from estrogen-treated female Heston A mouse 8 hours after injection of S^{35}-cystine. Somewhat tangential plane of section emphasizes zone of cystine concentration below stratum corneum. Stripping film, unstained. ×265.

FIG. 9.—Hair follicles from skin of male C_{57} mouse 8 hours after injection of 40 μc C^{14}-labeled algal protein-hydrolyzate. Note moderate activity of hair bulb extending into keratogenous zone. Stripping film (exposure 11 weeks), unstained. ×130.

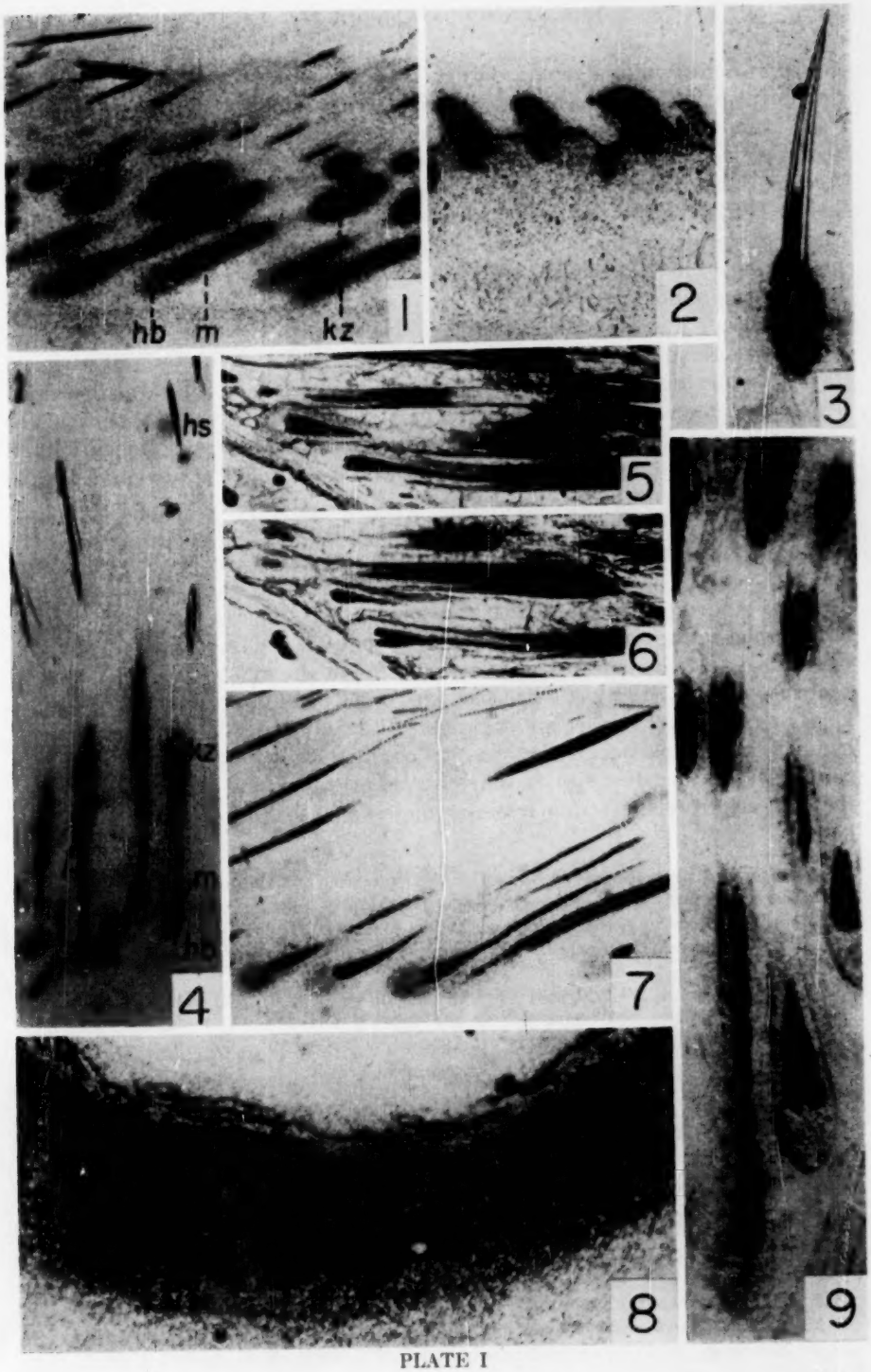

PLATE I

concentration of sulfur determined by chemical means in the stratum germinativum (Malpighian layer) is essentially the same as that in the stratum corneum in "soft" keratinization[7] could result from the fact that this cystine-concentrating zone (corresponding to the stratum granulosum-lucidum region) is included in the former. The results from our cystine studies would not support the suggestion of Leblond[2] that keratin itself may be the radiocarbon-tagged protein which moves "up with the cells of the skin towards the outside" (p. 472). Much of the cystine may be added to the precursor protein(s) (which presumably undergo prior hydrolytic decomposition[9]) just below the stratum corneum. In general, the evidence would indicate that keratinization is an active synthetic process, as Biggers' histochemical data indicated for the rodent vagina.[10]

The specific uptake of cystine by the keratogenous zone in hair formation raises some other interesting questions. The path of transport of the cystine from the circulatory system to the zone is not known. Absorption through the hair bulb is conceivable, but the possibility of a lateral transport from the capillaries around the follicle shaft[11] must also be considered.

Of great interest is the fact that the cells of the hair follicle which concentrate the cystine and synthesize the keratin evidently *de novo* are located well above the mitotically active hair bulb (in Hardy's zones D and E[11]). Hardy's extensive cytochemical studies[11] have shown that the spindle-shaped nuclei in the "moribund" cells of the keratogenous zone are still Feulgen-positive (DNA-containing) and that ribonucleic acid concentrations are also present in the cytoplasm. The residual protein-synthetic activity of cells which are "dying" and transforming thereby into keratin is worthy of further investigation. In avitaminosis-A, wherein squamous metaplasia and consequent keratinization occur in many epithelia, epithelial cells can be said to lose their specific differentiation capacity and their ability to synthesize specific proteins[12] while retaining the residual synthetic ability to produce keratin.

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*THE CEPHALOCARIDA, A NEW SUBCLASS OF CRUSTACEA FROM LONG ISLAND SOUND**

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In the course of a study of the benthic communities of Long Island Sound, eight individuals of a species of crustacean which could not be fitted into any of the known subclasses were discovered. The present paper is devoted to the description of their external anatomy. It is hoped that it will be the first of a series dealing with this most peculiar organism.

FAMILY HUTCHINSONIELLIDAE nov.

WITH CHARACTERS OF THE GENUS

Hutchinsoniella gen.nov.

Head large, horseshoe-shaped, produced backward over first thoracic segment as a short carapace, and bearing antennules, biramous antennae, palpless mandibles, and a single pair of biramous maxillae. Ten postcephalic appendages; first eight appendages biramous with at least a two-jointed protopodite bearing a large pseudepipodite on the basipodite and produced medially as a large complex gnathobase on all pairs; endopodite apparently six-jointed, exopodite two-jointed; ninth appendage lacking endopodite; tenth appendage reduced to two joints. Postcephalic segments 2–9 produced backwards at sides as well-developed pleura, segment 10 probably genital and with the succeeding nine abdominal segments bearing pleural spines. Telson with caudal furca armed with very long setose spines. Egg attached to tenth postcephalic segment of female. Generotype *Hutchinsoniella macracantha* sp.n.

Hutchinsoniella macracantha sp.nov.

Horseshoe-shaped head approximately as long as wide, making up 17–18 per cent of the total length of the animal excluding the caudal spines. Eyes absent. Labrum (Fig. 1, *C*) not produced backward greatly, covering a well-developed buccal cavity leading to an esophagus visible anteriorly.

Antennules (Fig. 2, *A*) with six wide basal joints and a thin terminal strap-shaped filamentous portion of about 35 joints. Distal surface of the second basal joint bearing a small knob apparently separated by an articulation (Fig. 2, *B*). The initial joint of the filament about equal to the length of the next four. A few hairs on and about the knob and larger cluster of long hairs present on the distal ends of the third and sixth basal segments of the antennules.

Antennae (Fig. 2, *C*) apparently made up of a two-jointed protopodite and two rami, one a two-jointed endopodite with the second segment rectangular in shape, the other a large club-shaped exopodite composed of about eighteen joints covered with numerous long hairs. Second segment of protopodite with a knoblike eminence bearing hairs (Fig. 2, *D*).

Mandibles without palp; median surface forming grinding molar plate covered with numerous tiny papillae and an incisor component of two spines with a movable bristle in between (Fig. 2, *E*).

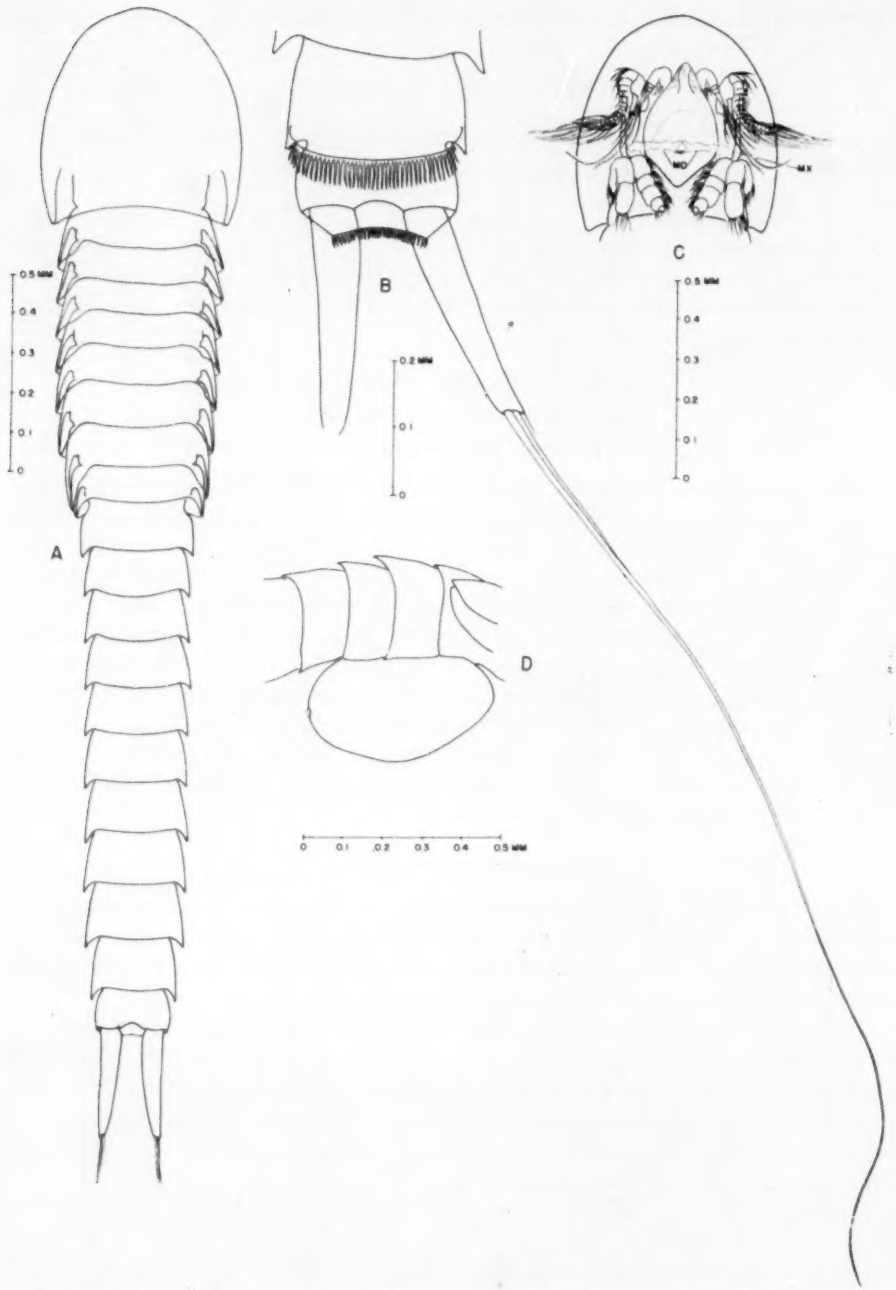

FIG. 1.—*A*, dorsal view of *Hutchinsoniella*. *B*, ventral view of posterior end of animal. *C*, ventral view of head region; *MD*, mandibles; *MX*, maxilla. *D*, lateral view showing egg attached to female.

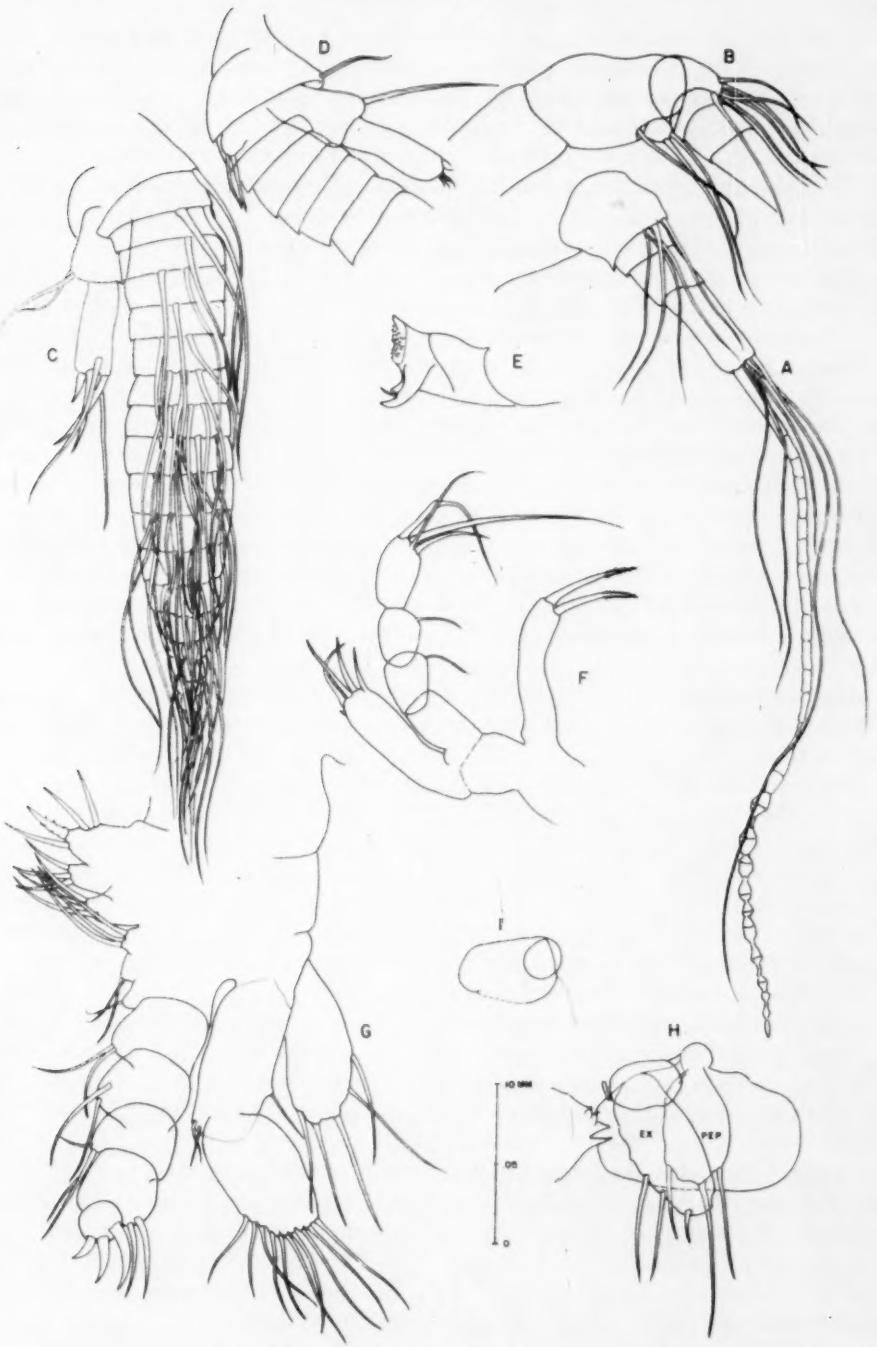

FIG. 2.—*A*, antennule with terminal part of filamentous portion twisted. *B*, basal section of antennule, showing small articulating knob. *C*, antenna. *D*, basal section of antenna, showing knoblike eminence. *E*, mandible. *F*, maxilla. *G*, first thoracic appendage. *H*, ninth thoracic appendage, showing interlocking processes of gnathobases; *EX*, exopodite; *PEP*, pseudepipodite. *I*, rudimentary tenth appendage.

Single pair of maxillae (Fig. 1, *C, MX;* Fig. 2, *F*) biramous and made up of a protopodite with an elongate gnathobase possessing two spines, the terminal portions of which are covered with fine hairs; a four-jointed endopodite with four terminal spines; a single-jointed exopodite with five terminal spines, one of which is very minute. (The homologies are tentative and subject to revision.)

First thoracic segment covered by carapace. Postcephalic segments 2–9 bear prominent pleura, and, together with thoracic segment 1, make up the thorax. On segments 10–18, the pleura represented by well-marked spines.

First seven thoracic appendages essentially similar. Protopodite at least two-jointed, with flattened paddle-like pseudepipodite derived from the basipodite on its lateral surface; a complex gnathobase of many lobes and spines on its medial margin. Exopodite two-jointed, flattened, the proximal joint being pentagonal and the distal joint circular in outline. A fringe of long spines on the distal edge of the second joint. Distal five joints of endopodite distinct, borne on a basal extension of the basipodite which may represent a sixth joint. The medial surface of the endopodite gives rise to numerous spines. The terminal joint round, smaller than the others, and giving rise to three short thick spines (Fig. 2, *G*). In segment 8 these three spines replaced by an elongate conical structure. The endopodite lost on ninth segment; the gnathobase can be clearly seen as shown somewhat schematically in Figure 2, *H*. On tenth segment the appendage greatly reduced, consisting merely of two joints (Fig. 2, *I*). In female this appendage used to hold egg case.

Genital segment probably tenth. No genital aperture discovered. Female bears single egg case, apparently containing a single large egg beneath 10–12 segments (Fig. 1, *D*).

Segments 11–18 lack appendages and may be considered to constitute the abdomen. The eighteenth segment bears a row of about forty-five equally spaced spines on distal ventral margin. A similar row of about twenty-eight smaller spines present on the distal ventral margin of telson. The posterior dorsal margin of telson excavated over anus. The telson gives rise to two elongate caudal furca, the rami of which are more than five times as long as wide. Each ramus bears three spines, two relatively small while the inner one is extremely long, being about one-half as long as total length of animal (Fig. 1, *B*).

Over-all length, excluding caudal spine, 2.85 mm.; width of abdomen, 0.33 mm.

Long Island Sound.—(1) Holotype, 2.85 mm. in length, stn. 8 (lat. 41-13.6; long. 72-46.4), July 23, 1954, on muddy bottom in 12–14 meters of water. Deposited in Peabody Museum of Yale University (Cat. No. 3617). (2) Paratype, 2.95 mm., stn. 3 (lat. 41-06.3; long. 73-00.2), August 20, 1954, on muddy bottom in 18–20 meters of water. (3) Paratype, female 2.30 mm. with egg case, stn. 8, August 1, 1953. (4) Paratype, female 2.80 mm. with remnants of egg case, stn. 7 (lat. 41-13.6; long. 72-50.6), September 10, 1954, on muddy bottom in 9–11 meters of water. (5) Paratype, 2.57 mm., stn. 7, May 20, 1954. (6) Paratype, 2.85 mm., stn. 8, July 23, 1954. (7) Paratype, dissected, stn. 8, July 23, 1954. (8) Paratype, dissected, stn. 3, December 23, 1953. All specimens collected by author.

It is not improbable that all the specimens are females.

It is apparent from the above description that *Hutchinsoniella* cannot be placed in any of the known subclasses as presently defined. It is therefore proposed that

a new subclass, Cephalocarida, be erected for this genus. The subclass may be defined as Crustacea with a large head posteriorly covering the first thoracic segment, with significantly more postcephalic segments than in the Malacostraca (which have fourteen or fifteen), with biramous thoracic appendages but no abdominal pleopods.

Discussion.—Regarding affinities, only three subclasses need be considered; the Branchiopoda, the Malacostraca, and the Copepoda. *Hutchinsoniella* possesses in common with the Branchiopoda the large number of thoracic and abdominal segments, the absence of pleopods on the abdomen, the presence of a furca, the mode of carrying the egg case, and the presence of gnathobases on all the thoracic appendages, although the rest of the appendage is totally different. However, its inclusion in the Branchiopoda must be ruled out by the form of the thoracic appendages, since they are far closer to the ideal biramous appendage even than the posterior thoracic appendages of the Devonian *Lepidocaris.*[1]

The most striking malacostracan character is the form of postcephalic appendages, which, except for the presence of an epipodite-like structure on the basipodite rather than the coxopodite, are comparable to those of the Syncarida. It is just possible that the knoblike structure on the second segment of the antennule represents a second antennulary ramus as in many Malacostraca. The number of segments and the absence of pleopods on the abdomen prevent *Hutchinsoniella* from being included in this subclass.

The large head, the method of carrying the egg, the absence of pleopods on the abdomen, and the shape of the caudal furca indicate affinities to the Copepoda. An extreme reduction of segments, some simplification of the appendages, and a fusion of the head to the first thoracic segment which it covers would convert *Hutchinsoniella* into an organism comparable to a copepod.

A peculiar feature of the animal is the presence of the pseudepipodite on the basipodite of the thoracic appendage. The Branchiura, however, possess a slender structure known as the flagellum, of doubtful homology,[2] that similarly is given off by the distal segment of the protopodite.

The second maxillae may have been lost, as in the cladocerans. It is, however, just conceivable that the first pair of thoracic appendages may be the homologue of the second pair of maxillae in other crustaceans.

The fact that the mandible lacks a palp may appear inconsistent with the general primitiveness of the animal. However, Borradaile[3] pointed out that among the decapods of the tribe Carides the palp disappears and reappears from genus to genus. At least in some groups of Crustacea the palp is evidently of little phylogenetic importance.

In conclusion, *Hutchinsoniella* may be looked upon as an extremely primitive crustacean at about the same level of complexity as the Branchiopoda but probably related to the ancestral stock that gave rise to Copepoda, Branchiura, and possibly the Malacostraca.

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aspects of this paper, and Miss Martha Dimock for aiding with the figures. Finally, Professor G. E. Hutchinson's insight and enthusiasm have been a constant source of encouragement, and the author is proud to name this organism in his honor.

* This work was aided by a contract between the Office of Naval Research, Department of the Navy, and Yale University, NR 163-118.

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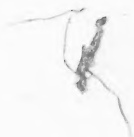

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